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BIOLOGICAL CONTROL OF THE COMMON ROOT ROT OF BARLEY THROUGH THE USE OF VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGI

by

(C) SUSAN MARY BOYETCHKO

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

PLANT PATHOLOGY

DEPARTMENT OF PLANT SCIENCE

EDMONTON, ALBERTA

FALL 1991

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"The progress of science is like an amoeba - advancing here, dragging there"

George W. Bruel, 1987

"in nature there are no families, no genera, no species.....
....there are only individuals more or less resembling one another"

Hochreutiner, 1929

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THE UNDERSIGNED CERTIFY THEY HAVE READ, AND RECOMMEND TO THE FACULTY OF GRADUATE STUDIES FOR ACCEPTANCE, A THESIS ENTITLED BIOLOGICAL CONTROL OF THE COMMON ROOT ROT OF BARLEY THROUGH THE USE OF VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGI SUBMITTED BY SUSAN MARY BOYETCHKO IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN PLANT PATHOLOGY.

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Abstract

Five species of VA mycorrhizal fungi were collected from agricultural soils in Alberta and identified. They included *Glomus mosseae, G. aggregatum, G. pansihalos, G. tenue*, and *Entrophospora infrequens*. *Glomus aggregatum* spores exhibited external proliferation, a condition reminiscent of microcyclic sporulation in other fungi. *Glomus pansihalos* is reported for the first time from western Canada. Scanning electron microscopy and energy dispersive X-ray microanalysis were used to examine spore surface morphology and relative calcium content of spore walls.

Field studies revealed that root colonization by indigenous VA mycorrhizal fungi was very low in 24 barley cultivars, with no significant differences among cultivars in susceptibility to these fungi. Greenhouse studies, however, revealed differences in root colonization by *Glomus dimorphicum, G. intraradices,* and *G. mosseae* in eight barley cultivars and differences in the ability of each *Glomus* species to colonize individual barley cultivars. Some, but not all, cultivars showed improved growth and yield by mycorrhizal fungi. However, the three *Glomus* species differed in their effectiveness to increase plant growth and yield.

Glomus dimorphicum, G. intraradices, and *G. mosseae* were effective biological control agents of barley common root rot, caused by *Bipolaris sorokiniana*, with *G. dimorphicum* being the least effective. The mechanisms involved in suppression of disease were investigated. Phosphorus application to soil reduced disease severity but not by as much as application of VA mycorrhizal fungi and phosphorus concommitantly. Phytoalexins were elicited in barley roots challenged by *B. sorokiniana* but could not be detected in roots colonized by mycorrhizal fungi. Perhaps, the concentration of phytoalexin elicited by VA mycorrhizal fungi was too low to be detected by the techniques used. Bonanza and Samson, cultivars moderately resistant to *B. sorokiniana*, elicited different amounts of phytoalexins, which may reflect different resistance mechanisms to disease in each cultivar. Susceptible cultivars, Olli and Galt, elicited lower levels of phytoalexin. Root exudates of barley cultivars showed no significant differences among cultivars in amounts of reducing sugars and amino acids. Negative correlations were observed between disease rating and exudation of reducing sugars and amino acids. However, no correlations were seen between susceptibility to VA mycorrhizal fungi and exudation of these compounds.

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Chapter I

Literature Review

A. Preface

Vesicular-arbuscular (VA) mycorrhizal fungi are symbiotic fungi forming associations with plant roots (Gerdemann, 1968; Mosse, 1973). Some benefits accrued to plants by this relationship are improved growth and yield as a result of increased uptake of nutrients such as phosphorus, zinc, copper and water (Gerdemann, 1968; Rhodes, 1980; Schenck, 1981; Hayman, 1983). The VA mycorrhizal fungi develop an extensive network of external hyphae which increase the absorbing capacity of plant roots by exploring a greater volume of soil normally inaccessible to the plant (see Fig. I-1) (Hayman, 1982; Gianinazzi-Pearson and Gianinazzi, 1983). Of particular importance is increased uptake of relatively immobile nutrients such as phosphorus, which become readily depleted around the root zone.

One other significant benefit to many plants is the suppression of plant diseases by VA mycorrhizal fungi (Schenck and Kellam, 1978; Schenck, 1981; Dehne, 1982; Hussey and Roncadori, 1982; Smith, 1988). Since VA mycorrhizal fungi are ubiquitous fungi found in almost all types of soils, it is common to observe these fungi and soil-borne plant pathogens on the same root (Smith, 1988). For the most part, studies of the interactions between VA mycorrhizal fungi and plant pathogens have concentrated on soil-borne fungi causing root rots and vascular wilts and plant-pathogenic nematodes. Further discussion in this chapter will consider these groups of pathogens only.

B. Classification and identification of VA mycorrhizal fungi

Recently, classification of VA mycorrhizal fungi was revised by Morton and Benny (1990). The criteria considered for the taxonomic revision of these fungi were based on evolutionary relationships in addition to spore morphology, ontogeny and germination (Morton, 1990; Morton and Benny, 1990). At present, VA mycorrhizal fungi are placed in the subdivision Zygomycotina, and in the new order Glomales Morton & Benny. Two suborders have been erected: suborder Glomineae Morton & Benny is comprised of the families Glomaceae Pirozynski &

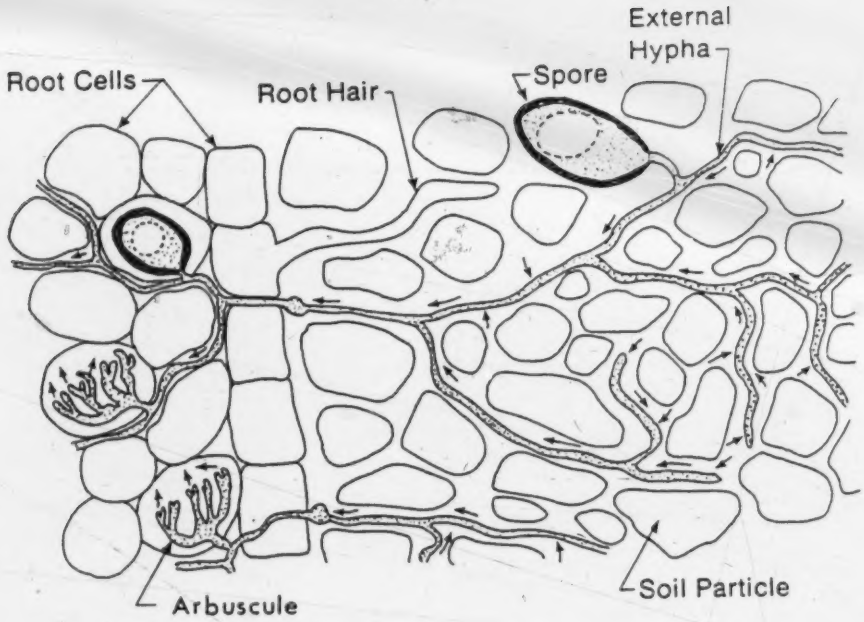

Figure I-1. Diagrammatic representation of the association of a VA mycorrhizal fungus with a plant root. Small arrows indicate the flow of nutrients such as phosphorus, zinc and copper into the root cortical cells. Not to scale.
From: Tewari, J.P., and S.M. Boyetchko. 1987.

Dalpé and Acaulosporaceae Morton & Benny while the suborder Gigasporineae Morton & Benny is comprised of the family Gigasporaceae (Morton and Benny, 1990). Two genera, *Glomus* Tul. & Tul. and *Sclerocystis* Berk. & Broome, are placed in the Glomaceae and are characterized by spores which are borne singly on one or more sporogenous hyphae (Morton and Benny, 1990). *Acaulospora* Gerd. & Trappe emend. Berch and *Entrophospora* Ames Schneider in the Acaulosporaceae typically develop spores laterally from or within a hypha which terminates in a sporiferous saccule (Morton and Benny, 1990). Finally, the family Gigasporaceae includes two genera, *Gigaspora* Gerd. & Trappe and *Scutellispora* Walker & Sanders. This group is characterized by the production of terminal spores on a sporogenous cell (Morton and Benny, 1990).

Criteria used to identify VA mycorrhizal fungi into species are predominantly based on sporocarp morphology, morphology of intact spores, including color and size, spore wall structure, morphology of the sporogenous hypha, and factors such as chemical and mechanical effects which modify the morphology of the spore (Morton, 1988). Examination of spore wall structure may reveal different types of ornamentation and number of spore walls, in addition to wall type. The wall type may be categorized as unit, laminate, evanescent, membranous (Walker, 1983), expanding (Berch and Koske, 1986), coriaceous (Walker, 1986) or amorphous (Morton, 1986). Not all mycorrhizal species contain all wall types. Walker (1983) suggested that mycorrhizal taxonomists adopt the use of graphical representations of wall structures (murographs) to use as standardized diagrams to facilitate study of spore wall characteristics. A summary of murographs of spore walls of VA mycorrhizal fungal species are provided by Morton and Benny (1990).

Reaction of spore walls to Melzer's reagent offers a useful means for identifying VA mycorrhizal fungi. This reagent is comprised mostly of iodine which reacts with certain components in the spore wall to produce various color reactions (Morton, 1988). The different colors represent the chemical composition of the spore wall and the extent of polymerization of these compounds. Mountants used to preserve spores on glass slides can affect spore morphology. Phenol-based mountants will darken spore walls while lactic acid may affect staining reactions to Melzer's reagent or elicit the expanding wall seen in *Glomus pansihalos* Berch and Koske (1986).

Another technique previously used to characterize components in VA mycorrhizal fungal spore walls was energy dispersive X-ray microanalysis (Boyetchko and Tewari, 1986). This method was employed to detect calcium in the spore walls of *Glomus dimorphicum* Boyetchko & Tewari. It is possible that this technique may be useful to determine the elemental composition of VA mycorrhizal spore walls.

Other useful techniques which may be employed in the future to identify VA mycorrhizal fungi are isozyme studies using polyacrylamide gel electrophoresis (Sen and Hepper, 1986; Hepper *et al.*, 1988), immunological studies such as enzyme-linked immunosorbent assay (ELISA) (Aldwell *et al.*, 1983) and nucleotide sequencing (Morton, 1988). A major criticism of these techniques, at present, is that no relationship with morphological characteristics has been established. Extensive research is necessary before these techniques can be incorporated as criteria for the identification of VA mycorrhizal fungi.

C. Biocontrol of diseases caused by soil-borne fungi

The first report describing the interaction between a VA mycorrhizal fungus and a plant pathogenic fungus was documented by Safir (1968). There have since been numerous studies on the interaction between VA mycorrhizal fungi and soil-borne plant pathogens (see Table I-1).

Generally, results have shown significant decreases in disease severity (Davis and Menge, 1980; Graham and Menge, 1982; Zambolin and Schenck, 1983; Caron *et al.*, 1986a, 1986b). Schönbeck and Dehne (1977) reported that mycorrhizal cotton seedlings were able to tolerate damage caused to roots by *Thielaviopsis basicola* (Berk. & Broome) Ferraris better than were nonmycorrhizal cotton seedlings. The disease occurred at the same extent on both mycorrhizal and nonmycorrhizal plants, but the precise mechanism for disease tolerance was not explained. Baltruschat and Schönbeck (1972) noted that chlamydospore production by *T. basicola* on tobacco roots was greatly reduced in mycorrhizal plants infected with the pathogen. Extracts obtained from mycorrhizal roots inhibited production of the chlamydospores by 80 to 100%. However, the nature of the compounds in the mycorrhizal root extracts was not examined. Perhaps the VA mycorrhizal fungi induced the production of such compounds as isoflavonoids, which are known to be inhibitory to certain fungi (Morandi *et al.*, 1984).

TABLE I - 1. Influence of VA Mycorrhizal Fungi on Soil-Borne Fungal Pathogens

Host Plant	Pathogen	Effect of VAM on Disease[a]	Reference
Alfalfa	*Phytophthora megasperma*	+	Davis *et al.* 1978
	Pythium paroecandrum	-	Hwang 1988
Barley	*Bipolaris sorokiniana*	-	Grey *et al.* 1989; Thompson & Wildermuth 1989
Citrus	*Phytophthora parasitica*	-	Davis & Menge 1980, 1981
	P. parasitica	+	Davis *et al.* 1978
	Thielaviopsis basicola	0	Davis 1980
Cotton	*T. basicola*	-	Schönbeck & Dehne 1977
	Verticillium dahliae	+	Davis 1980
Onion	*Pyrenochaeta terrestris*	-	Safir 1968
Peanut	*Sclerotium rolfsii*	-	Krishna & Bagyaraj 1983
Poinsettia	*Pythium ultimum*	-	Kaye *et al.* 1984; Stewart & Pfleger 1977
	Rhizoctonia solani	-	Stewart & Pfleger 1977
Soybean	*Fusarium solani*	-	Zambolin & Schenck 1983
	Macrophomina phaseolina	-	Zambolin & Schenck 1983
	P. megasperma	-	Chou & Schmittenhenner 1974; Schenck 1981
	R. solani	-	Zambolin & Schenck 1983
Strawberry	*Phytophthora fragariae*	0	Bååth & Hayman 1984
Tobacco	*T. basicola*	-	Baltruschat & Schönbeck 1972
Tomato	*Fusarium oxysporum* f.sp. *lycopersici*	-	Dehne & Schönbeck 1975, 1979a, 1979b; Caron *et al.* 1985, 1986a, 1986b, 1986c
	Verticillium albo-atrum	0	Bååth & Hayman 1983
Wheat	*Gaeumannomyces graminis tritici*	-	Graham & Menge 1982

a 0 = no effect, + = increase, - = decrease

Studies have shown that the presence of VA mycorrhizal fungi reduced the damage by *Phytophthora megasperma* Drechs. on soybean (Chou and Schmitthenner, 1974; Schenck, 1981). *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe did not affect disease severity but did reduce the number of plants killed by the pathogen. It is unknown what the mechanism of disease suppression by the mycorrhizal fungi was. No information was provided on the soil nutrient levels or whether one or more phosphorus levels were used in the study. Another study involving soybean was conducted by Zambolin and Schenck (1983). The results showed that the presence of *G. mosseae* in roots infected by *Macrophomina phaseolina* (Tassi) Goid., *Rhizoctonia solani* Kuhn, and *Fusarium solani* (Mart.) App. & Wr. emend. Snyd. & Hans. significantly improved plant growth over plants not colonized by the mycorrhizal fungus. The infection levels of the pathogens in the roots was similar in both mycorrhizal and nonmycorrhizal plants and it was believed that mycorrhizal plants tolerated infection by pathogens more than nonmycorrhizal plants. Although improved phosphorus uptake by *G. mosseae* was suggested as a possible mechanism for improved tolerance of the plants to the pathogens, the levels of soil phosphorus used in the study were quite adequate to support normal growth of soybean.

Hwang (1988) observed a reduction in severity of alfalfa sickness caused by *Pythium paroecandrum* Drechsler due to VA mycorrhizal fungi. The results indicated that the introduction of VA mycorrhizal fungi to the soil caused a stimulation of alfalfa growth even in the presence of the pathogen. The soil used in the study contained 20.8 ppm available phosphorus. The author did not determine whether the mechanism for disease resistance was improved phosphorus uptake or a greater tolerance of mycorrhizal plants to the pathogen. Mycorrhizal poinsettia plants also have been shown to tolerate infection by *Pythium ultimum* Trow (Kaye *et al.*, 1984). The tolerance was thought to be caused by the reduction of inoculum density of *P. ultimum* in the rhizosphere of mycorrhizal plants relative to nonmycorrhizal plants. Mycorrhizal plants had greater concentrations of phosphorus in the leaves than did nonmycorrhizal plants. Reduced root exudation resulting from improved phosphorus uptake may have had an effect on reducing the inoculum density of *P. ultimum*. However, when inoculum levels of the pathogen applied to the soils were highest (132 cfu/g root), no significant reduction in inoculum density of *P. ultimum* by VA mycorrhizal fungi was observed. These results revealed that extremely high initial inoculum levels of the pathogen may

nullify any beneficial effect of the mycorrhizal fungi and should caution other researchers to use various levels of pathogen inoculum in their studies. In citrus, tolerance to Phytophthora root rot was observed in plants inoculated with different VA mycorrhizal species (Davis and Menge, 1980, 1981). The citrus was exposed to VA mycorrhizal fungi before introducing the plants to the pathogen. The results showed that mycorrhizal citrus plants contained more phosphorus than nonmycorrhizal citrus plants and tolerated root rot caused by low levels of *Phytophthora parasitica* Dast. inoculum (Davis and Menge, 1980). A later study revealed that different species of VA mycorrhizal fungi differed in their ability to improve growth of citrus (Davis and Menge, 1981). Those species which were more efficient at improving phosphorus uptake by the plant conferred greater tolerance to Phytophthora root rot. Growth of mycorrhizal citrus plants infected by the pathogen was greater than nonmycorrhizal citrus plants infected by the pathogen, although disease levels were higher in mycorrhizal than nonmycorrhizal citrus plants (Davis and Menge, 1981). VA mycorrhizal fungal species not as nutritionally efficient elicited a resistance mechanism by the host which eventually caused a reduction of infection by *P. parasitica* (Davis and Menge, 1981). In this case, the VA mycorrhizal fungal species involved did not produce significant growth improvements over nonmycorrhizal plants nor did the fungi improve phosphorus uptake. Although disease levels were lower in mycorrhizal plants than nonmycorrhizal plants, the precise mechanism for resistance was not elucidated. Changes in plant physiology as a result of mycorrhizal symbiosis may be involved and further work in the area of mechanisms for disease resistance should provide a more complete explanation for the results seen in the study.

In cereal crops, the presence of *Glomus* Tul. & Tul. species in the soil led to reduced severity of barley common root rot (Grey *et al.*, 1989; Thompson and Wildermuth, 1989; Rempel and Bernier, 1990) and take-all disease of wheat (Graham and Menge, 1982). Grey *et al.* (1989) observed a reduction in the number of subcrown internodes of barley showing lesions infected with *Bipolaris sorokiniana* (Sacc.) Shoem. when VA mycorrhizal fungi were added to soil. Comparison of two barley cultivars revealed that mycorrhizal fungi improved yield of one cultivar, infected with the pathogen, while the other cultivar showed no improvement in growth. These results illustrated that different cultivars may react in different manners to VA mycorrhizal fungi. Grey *et al.* (1989), however, did not indicate the initial inoculum density of the pathogen or the species of VA mycorrhizal fungus used in the study. These factors also have an effect on the

outcome of the experiment. Thompson and Wildermuth (1989) also provided information which showed an inverse relationship between VA mycorrhizal root colonization and infection of roots by *B. sorokiniana*. However, information on initial levels of conidia of *B. sorokiniana* or spores of VA mycorrhizal fungi in the soil were not provided. It appeared that the authors simply compiled data obtained from fields in which winter and summer crops were grown. No cause and effect relationship between the pathogen and mycorrhizal symbiont was established. Graham and Menge (1982) showed that the reduction of take-all disease severity was mediated by improved phosphorus nutrition. As plant phosphorus increased, a corresponding decrease in exudation of amino acids and reducing sugars by roots was observed (Graham and Menge, 1982). A correlation was observed between increased levels of root exudates and a reduction in root phospholipids in phosphorus-deficient soils.

Several studies have been conducted on the suppression of *Fusarium oxysporum* Schlecht f.sp. *lycopersici* Jarvis & Shoemaker on tomato. A significant reduction in wilting caused by the pathogen was observed in tomato plants inoculated with *G. mosseae* (Dehne and Schönbeck, 1975; 1979a). The spread of the pathogen within the plant was limited and chlorophyll content was greater in mycorrhizal plants than in nonmycorrhizal plants. Further studies revealed an increase in lignin deposition of plant cell walls which restricted spread of Fusarium wilt (Dehne and Schönbeck, 1979b). It is not clear, though, if the quantity of lignin produced could be sufficient to explain resistance. Other studies investigated the effect of *G. intraradices* Schenck & Smith on crown and root rot caused by *F. oxysporum* f.sp. *radicis-lycopersici* (Caron *et al.*, 1985; 1986a, 1986b). The interaction between the pathogen and VA mycorrhizal fungus was monitored over a 12-week period (Caron *et al.*, 1986a). The mechanism for suppression of the disease was found to be stable over time. The results also revealed that propagules of *F. oxysporum* f.sp. *radicis-lycopersici* were reduced when plants were inoculated with *G. intraradices* . The authors had previously reported that the disease development was influenced by the substrate used during the course of the experiment (Caron *et al.*, 1985). It was suggested that a substrate such as calcined montmorillonite clay, a soilless substrate, be used as a standard medium to study interactions between VA mycorrhizal fungi and root pathogens. Use of an artificial medium may, however, lead to different results from those obtained by using the substrate used in normal practices by the grower. A study on Fusarium crown and root rot and *G. intraradices* in a soilless substrate revealed

that increasing levels of phosphorus had no effect on shoot growth, necrosis of roots caused by the pathogen, or the final propagule density of *F. oxysporum* f.sp. *radicis-lycopersici* (Caron *et al.*, 1986b). However, no reason was offered for the observed reduction in disease development at all levels of phosphorus, even though VA mycorrhizal root colonization was reduced as the application of phosphorus to the soil increased. Graham and Menge (1982) suggested that a certain level of root colonization by VA mycorrhizal fungi is required to influence the effect of a pathogen on the host. Increasing the level of phosphorus available to the plant affected the influence of *G. fasciculatum* (Thaxter sensu Gerd.) Gerd. & Trappe on the severity of take-all disease of wheat (Graham and Menge, 1982).

Some studies have revealed either an increase (Ross, 1972; Davis *et al.*, 1978; 1979) or no effect (Davis, 1980; Bååth and Hayman, 1983; 1984) on severity of diseases due to infection by VA mycorrhizal fungi. Ross (1972) was the first to report an increase in disease severity due to *G. macrocarpum* var. *geosporum* Tul. & Tul. Phytophthora root rot of soybean was more severe in mycorrhizal plants than in nonmycorrhizal plants. The results suggested that the production of large vesicles and chlamydospores by *G. macrocarpum* damaged the host tissue and hence created a means for the pathogen to enter and develop inside the plant tissue. Davis *et al.* (1978) tested the ability of *G. fasciculatum* to suppress Phytophthora root rot in citrus, avocado, and alfalfa. They discovered that *Phytophthora* caused a significant reduction in growth of both mycorrhizal and nonmycorrhizal alfalfa seedlings. However, mycorrhizal avocado and citrus plants were affected more by *Phytophthora* than those that were nonmycorrhizal. It was believed that *Phytophthora* may reduce the inoculum potential and thus root colonization of *G. fasciculatum*. No direct evidence was provided to support this argument, however. It was only observed that phosphorus levels were similar in mycorrhizal and nonmycorrhizal avocado and citrus plants infected by *Phytophthora*. It was suggested that the effect of VA mycorrhizal fungi on soil-borne fungal pathogens varied with the disease complex but further explanation for these results were lacking. Verticillium wilt of cotton was also more severe when plants were inoculated with *G. fasciculatum* (Davis *et al.*, 1979). Adequate levels of phosphorus nutrition due either to application of phosphorus fertilizer or improved phosphorus uptake by VA mycorrhizal fungi resulted in greater severity of Verticillium wilt than in plants grown in soils low in phosphorus. It was suggested that improved plant phosphorus nutrition supported a larger population of *Verticillium dahliae* Kleb. resulting in an increase in disease severity.

No effect of VA mycorrhizal fungi was observed in citrus infected with *T. basicola* (Davis, 1980), strawberry infected with *Phytophthora fragariae* Hickman (Bååth and Hayman, 1984) or tomato infected with *Verticillium albo-atrum* Reinke & Berthoid (Bååth and Hayman, 1983). The relative growth reduction by *T. basicola* in mycorrhizal and nonmycorrhizal citrus plants was the same, although the mycorrhizal seedlings were larger than nonmycorrhizal seedlings in soil infested with the pathogen (Davis, 1980). Bååth and Hayman (1984) also observed no significant effect of *Glomus* species on red core disease, caused by *P. fragariae* . The authors concluded that VA mycorrhizal fungi may interact differently with various *Phytophthora* species and strains. The susceptibility of the cultivar and the mode of pathogenesis and aggressiveness of the pathogen could influence the effect that the VA mycorrhizal fungus has on the disease and this must be considered during the course of any study on interactions between soil-borne pathogens and mycorrhizal fungi. Bååth and Hayman (1983) made similar conclusions in relation to Verticillium wilt of tomato.

D. Biocontrol of diseases caused by soil-borne nematodes

Nematodes and VA mycorrhizal fungi occur in the rhizosphere and roots of plants and have significant influence on plant health. Rich and Schenck (1981) reported a positive correlation between mycorrhizal spore numbers and nematode populations. This observation indicated that not only do these two groups of organisms coexist spatially and temporally, but that there is increasing probability that they may interact with one another. The first observation on the interaction between these organisms was reported by Fox and Spasoff (1972). It was found that *Endogone gigantea* Nicol. & Gerd. reduced the population of *Heterodera solanacearum* Miller and Gray in tobacco. Several studies have since reported the various interactions between VA mycorrhizal fungi and plant parasitic nematodes (see Table I-2). In the majority of cases, studies have concentrated on sedentary endoparasites while other reports have involved interactions with migratory endoparasites (Hussey and Roncadori, 1982; Bagyaraj, 1984).

Two types of effects on plant growth influenced by the interaction between VA mycorrhizal fungi and nematodes may occur. In the Type I response, the growth stimulation of the plant as a result of the VA mycorrhizal fungus is reduced by the nematode (Ingham, 1988). However, the growth of the plant, when infected

Table I - 2. Influence of VA Mycorrhizal Fungi on Soil-Borne Nematodes

Host Plant	Pathogen	Effect on Disease[a]	Effect on Nematode Reproduction[b]	Reference
Cantaloupe	*Meloidogyne incognita*	-	?	Heald *et al.* 1989
Citrus (lemon)	*Radopholous citrophilus*	-	-	Smith & Kaplan 1988
	R. similis	0	-	O'Bannon & Nemec 1979
	Tylenchulus semipenetrans	-	0	O'Bannon *et al.* 1979
Cotton	*M. incognita*	-	+	Roncadori & Hussey 1977
	M. incognita	-	-	Saleh & Sikora 1984
				Grandison & Cooper 1986
				Smith *et al.* 1986b
	Pratylenchus brachyurus	-	0	Hussey & Roncadori 1978
Grape	*Meloidogyne arenaria*	-	+	Atilano *et al.* 1981
Onion	*Meloidogyne hapla*	-	0	MacGuidwin *et al.* 1985
Peach	*M. incognita*	-	-	Hussey & Roncadori 1982
	M. incognita	-	?	Strobel *et al.* 1982
Peanut	*M. arenaria*	-	+	Hussey & Roncadori 1982
Soybean	*Heterodera gycuines*	-	+	Francl & Dropkin 1985
	M. incognita	-	-	Kellam & Schenck 1980
				Carling *et al.* 1989
Tamarillo	*M. incognita*	-	-	Cooper & Grandison 1987
Tobacco	*Heterodera solanacearum*	?	-	Fox & Spasoff 1972
Tomato	*M. hapla*	-	-	Cooper & Grandison 1986
	M. incognita	?	-	Bagyaraj *et al.* 1979
	M. incognita	-	-	Suresh *et al.* 1985
	M. incognita	-	0	Thomson *et al.* 1983
	Meloidogyne javanica	?	-	Bagyaraj *et al.* 1979
	Rotylenchulus reniformis	-	-	Sitaramaiah & Sikora 1982

a,b. 0 = no effect, + = increase, - = decrease, ? = unknown

by both the nematode and mycorrhizal fungus, is still higher than the control (which lacks the presence of both organisms). In the Type II response, the presence of the VA mycorrhizal fungus compensates for the reduction in plant growth caused by the nematode (Ingham, 1988), although the plant growth or yield is higher in the control plants than in plants which are concomitantly infected by the nematodes and VA mycorrhizal fungus. Ingham (1988) found that more interaction studies fall within the Type I response than in the Type II response.

1. Sedentary endoparasitic nematodes

The population dynamics of sedentary endoparasitic nematodes can be affected by VA mycorrhizal fungi. The rate of penetration and/or development of the nematode may be influenced by the presence of the mycorrhizal symbiont (Dehne, 1982). Most interaction studies between VA mycorrhizal fungi and sedentary nematodes have been conducted with root knot nematodes belonging to the genus *Meloidogyne* Goeldi. The total reproduction rate of the nematode may be increased but damage to mycorrhizal plants may be lower than nonmycorrhizal plants.

The presence of *Meloidogyne arenaria* (Neal) Chitwood in grape reduced the effect of *G. fasciculatum* on plant growth when the nematode inoculum applied was low (approx. 200 eggs/plant). Furthermore, the final nematode population was highest in mycorrhizal plants (Atilano *et al.*, 1981). Hussey and Roncadori (1982) also observed an increase in reproduction of *M. arenaria* in peanut colonized by either *G. etunicatum* Becker & Gerd. or *Gigaspora margarita* Becker & Hall. Although there was an eight-fold increase in the number of eggs per gram root, the presence of the nematode had no negative effect on the improved plant growth caused by the mycorrhizal fungi. Roncadori and Hussey (1977) observed no significant effect of *G. margarita* on nematode reproduction but found a significant increase in the total number of *Meloidogyne incognita* (Kofoid & White) Chitwood eggs per plant in the mycorrhizal cotton plants. They attributed the increase in number of eggs to the increase in growth of mycorrhizal roots over nonmycorrhizal roots. Soybeans colonized by an isolate of *G. fasciculatum* had fewer first generation cyst nematodes (*Heterodera glycines* Ichinohe) than nonmycorrhizal soybeans (Francl and Dropkin, 1985). However, the number of second generation females on mycorrhizal roots was significantly greater than on nonmycorrhizal roots. The increase in plant biomass influenced by

G. fasciculatum supported the development of a greater number of nematodes than nonmycorrhizal plants. The greater plant root biomass may have provided more sites of penetration available to the nematodes. In a split-root experiment, numbers of eggs per plant and numbers of eggs per gram root of *M. incognita* were increased when *G. margarita* was on the same or opposite side of the root system (Strobel *et al.*, 1982). However, the results observed throughout the study were variable and the authors could not account for the inconsistent effects of the mycorrhizal fungus on nematode reproduction. It is possible that VA mycorrhizal fungi may induce the plant to produce compounds inhibitory to the nematodes. Some of these compounds may even be translocated to other parts of the root. Extensive research is required, though, to study such a possibility.

Other studies have reported no effect of VA mycorrhizal root colonization on nematode populations. *Glomus etunicatum* infecting peach had no effect on *M. incognita* reproduction (Strobel *et al.*, 1982) and the density of *M. hapla* Chitwood was not affected by *G. fasciculatum* in onion (MacGuidwin *et al.*, 1985). It was postulated that *G. fasciculatum* altered the host physiology which in turn impaired the penetration of *M. hapla* in onion roots. These conclusions were supported by evidence that nonmycorrhizal plants supplemented with phosphorus had lower penetration rates of *M. hapla* than nonmycorrhizal plants not supplemented with phosphorus. Thomson Cason *et al.* (1983) also observed no effect on penetration and reproduction of *M. incognita* in tomato by either *G. margarita* or *G. mosseae* . Supplemental phosphorus altered nematode infection more than the VA mycorrhizal fungi and it was suggested that the mycorrhizal fungi probably enhanced plant tolerance to *M. incognita* , thus offsetting the damage caused by the nematode. The high phosphorus application (30 µg available phosphorus per gram soil) may have had a negative effect on the ability of mycorrhizal symbionts to reduce nematode infection. An intermediate level of phosphorus may elucidate whether very high soil phosphorus levels negated the beneficial effect of the VA mycorrhizal fungi.

For the most part, nematode reproduction is reduced on certain hosts which are colonized by VA mycorrhizal fungi. Preinoculation of tomato with VA mycorrhizal fungi at least four weeks before inoculation with *M. hapla* led to reduced numbers of nematodes (Cooper and Grandison, 1986). Adult nematode development was suppressed and the number of juveniles per gram of root was always lower in mycorrhizal plants than nonmycorhizal plants. The plant's resistance to *M. hapla* was also increased due to VA mycorrhizal fungi. Similar

results were observed in peach seedlings infected with *M. incognita* (Hussey and Roncadori, 1982) and soybeans infected with *M. incognita* (Carling *et al.*, 1989). Preinoculation of plants with VA mycorrhizal fungi yielded better suppression of diseases caused by nematodes than simultaneous inoculation of the two organisms (Hussey and Roncadori, 1982; Cooper and Grandison, 1986; 1987). Although inoculation of plants with VA mycorrhizal fungi prior to inoculation of plants with a pathogen may favor the mycorrhizal fungus and represent an artificial system, preinoculation of containerized or transplantable crops may be a realistic approach for controlling nematodes. Use of several species of VA mycorrhizal fungi at the same time also provided maximum protection and resistance to nematodes (Cooper and Grandison, 1987). Most soils contain a few to several species of VA mycorrhizal fungi and would represent a realistic scenario.

Sitaramaiah and Sikora (1982) observed fewer juveniles of *Rotylenchulus reniformis* Linford & Oliveira in tomato preinoculated with *G. fasciculatum* than nonmycorrhizal control plants. The nematode juveniles penetrated the tomato roots less in mycorrhizal plants than nonmycorrhizal plants. The rate of development from juvenile to egg-laying females was reduced in mycorrhizal plants (MacGuidwin *et al.*, 1985; Smith *et al.*, 1986b). Significant reductions in body width of females and number of eggs per egg sac were also observed in plants colonized by VA mycorrhizal fungi (Sitamaraiah and Sikora, 1982). Other researchers have further observed an absence of nematodes in root cortical tissue containing a certain level of VA mycorrhizal colonization (Saleh and Sikora, 1984; Grandison and Cooper, 1986; Smith *et al.*, 1986b). Smith *et al.* (1986b) found that *M. incognita* was not affected by 20-30% root colonization by *G. intraradices* in cotton. When 50% of the root was colonized by the fungus, the nematodes were inhibited. Saleh and Sikora (1984), however, observed that numbers of *M. incognita* were not reduced until root colonization by *G. fasciculatum* was 80% or more, while Grandison and Cooper (1986) found that nematodes were absent from root cortical tissue with more than 10% VA mycorrhizal colonization. Smith *et al.* (1986b) suggested that competition for plant photosynthate or even production of nematicidal compounds by VA mycorrhizal fungi was more important than competition for space since the authors still observed a reduction in nematodes when at least half the root system was yet uncolonized by VA mycorrhizal fungi. Future research should consider the possibility that nematicidal compounds may be translocated systemically to other parts of the plant root, thus exerting an influence beyond the site of VA mycorrhizal fungal structures.

Some nematodes, such as *Meloidogyne* species, induce the formation of hypertrophic tissue which forms galls. Associated with the hypertrophied tissue are giant cells which develop in response to nematode infection and serve as transfer cells for the flow of nutrients towards the nematode (Dehne, 1982; Agrios, 1988). Kellam and Schenck (1980) revealed that *G. macrocarpum* reduced the number of galls and giant cell development of *M. incognita* in soybean. *Glomus fasciculatum* in tomato also decreased the number of nematode galls produced by *M. incognita* and *M. javanica* (Trent) Chitwood (Bagyaraj *et al.*, 1979). It was also found that plants containing only nematodes developed many large multiple galls while plants colonized with VA mycorrhizal fungi and nematodes, developed small primary galls. Suresh *et al.* (1985) found that fewer giant cells developed in each gall caused by *M. incognita* and that the average cell size was less in mycorrhizal than in nonmycorrhizal roots. Other studies showed that VA mycorrhizal fungi were not present in the vicinity of nematode galls (Heald *et al.*, 1989) while in other cases, vesicles and arbuscules were found in nematode galls but not in giant cells (Hussey and Roncadori, 1982). Kellam and Schenck (1980) observed that VA mycorrhizal fungal structures appeared to decompose within the nematode gall, while structures above or below the gall appeared normal. Generally, evidence has indicated that VA mycorrhizal fungi do not colonize tissue which is already inhabited by nematodes. On the other hand, nematodes may infect root tissues already colonized by VA mycorrhizal fungi (Ingham, 1988). It is, however, difficult to determine from many reports whether the nematode or the fungus was the first to colonize a root area.

2. Migratory endoparasitic nematodes

Less information is available concerning interactions between VA mycorrhizal fungi and migratory nematodes than sedentary nematodes. Total numbers of *Pratylenchus brachyurus* (Godfrey) Filip. & Schistek. did not differ between mycorrhizal and nonmycorrhizal cotton plants, but the density of nematodes per gram of root was less in cotton colonized by *G. margarita* (Hussey and Roncadori, 1978). As with some sedentary nematodes, less damage due to nematodes was the result of a dilution effect from the larger root systems attributed to the VA mycorrhizal fungi. The authors also observed that the majority of nematodes were found within roots that were nonmycorrhizal and in rare cases were the nematodes noticed in the root cortical tissue inhabited by the VA

mycorrhizal fungus. It was suggested that the nematodes competed for sites otherwise occupied by the symbiont. O'Bannon and Nemec (1979) observed that reproduction of *Radopholus similis* (Cobb) Thorne was similar on mycorrhizal and nonmycorrhizal rough lemon seedlings. However, seedlings inoculated with *G. etunicatum* before inoculation with the nematode were significantly larger than seedlings inoculated with nematodes alone. Studies on *Tylenchulus semipenetrans* Cobb indicated that mycorrhizal root colonization had no effect on the nematode population (O'Bannonn *et al.*, 1979). In addition, the VA mycorrhizal fungus compensated for the loss in growth of rough lemon caused by *T. semipenetrans* by improving the plant's tolerance to the nematodes. Because this study was conducted in phosphorus-deficient soils, it is not known whether increased tolerance may have resulted from improved host nutrition of the mycorrhizal plants. Smith and Kaplan (1988) revealed that phosphorus played a role in decreasing damage from *Radopholus citrophilus* Huettel in mycorrhizal rough lemon plants. Mycorrhizal plants had similar nematode populations, plant growth and phosphorus concentrations as nonmycorrhizal plants when phosphorus was applied at a rate of 300 mg phosphorus per kg soil. However, when only 25 mg phosphorus per kg soil was applied, the nematode densities were less and plant growth and phosphorus nutrition were greater in mycorrhizal plants than nonmycorrhizal plants.

In most studies, the nematodes did not affect root colonization by VA mycorrhizal fungi (Hussey and Roncadori, 1982; Bagyaraj, 1984). Sporulation by VA mycorrhizal fungi was variable in the presence of nematodes. In some cases, fewer spores were observed while in others, no differences in sporulation were found (Hussey and Roncadori, 1982).

E. Mechanisms of suppression of disease

The theories used to explain the mechanism by which VA mycorrhizal fungi reduce the severity of disease caused by soil-borne pathogens have either a physiological or physical basis (Dehne, 1982; Graham, 1988). In general, VA mycorrhizal fungi affect the relationship between the host and pathogen by physiologically altering the host or by competing with the pathogen for space or host photosynthate. Nutritional benefits accrued by VA mycorrhizal symbiosis, as well as induced biochemical alterations of the plant, provide a basis for further

theories concerning the indirect effects of VA mycorrhizal fungi on soil-borne pathogens.

1. Nutritional effects

Improved plant phosphorus nutrition is one of the most popular theories used to explain the suppression of disease mediated by VA mycorrhizal fungi. The increase in phosphorus uptake by plants due to mycorrhizal fungi is thought to lead to reduced severity of diseases caused by soil-borne fungi and nematodes (Davis and Menge, 1980; Graham and Menge, 1982; Smith *et al.*, 1986b; Carling *et al.*, 1989). The increased tolerance of mycorrhizal and phosphorus-fertilized plants led to larger root systems which thus led to tolerance of plants to nematodes (Hussey and Roncadori, 1982; Smith *et al.*, 1986b). Graham and Menge (1982) explained the decrease in take-all disease by an increase in phosphorus nutrition as a result of increased phosphorus uptake by *G. fasciculatum* . Along with improved plant phosphorus nutrition was a corresponding reduction in exudation of reducing sugars and amino acids from roots. Levels of phosphorus in plants appeared to regulate root exudation through the effect on phospholipids which are associated with root membrane permeability (Ratnayake *et al.*, 1978; Graham *et al.*, 1981). Root exudation exerts an influence on soil-borne pathogens where phosphorus-induced decreases in root exudation may result in lower activity of soil-borne pathogens and thus decreases in severity of disease. Changes in root exudation may also affect the hatching of nematodes from eggs in certain nematode species which require a hatching stimulus (Graham, 1988). Future studies should also be conducted to determine whether VA mycorrhizal fungi cause the exudation of compounds inhibitory to soil-borne pathogens into the rhizosphere.

High levels of soil phosphorus can interact with the uptake of minor elements such as zinc, thus creating deficiencies and predisposing the plant to soil-borne nematodes (Smith *et al.*, 1986a). Phosphorus-induced zinc deficiency led to greater damage of cotton by *M. incognita* . It is believed that mycorrhizal fungi play an important role in absorption of nutrients, such as phosphorus, zinc and copper, which are are not very mobile in soil. In general, VA mycorrhizal root colonization decreases when soil phosphorus levels are high. This may lead to a reduction in mycorrhizal root colonization and therefore a reduction in uptake of the less mobile nutrients which are normally present in low concentrations in soil solution (Gianinazzi-Pearson and Gianinazzi, 1983). Application of mycorrhizal

fungi may improve the plant's tolerance to pathogens by increasing the uptake of such elements as zinc and copper, besides phosphorus, which would otherwise be deficient in nonmycorrhizal plants.

2. Competition for host photosynthate, space and infection site

VA mycorrhizal fungi are dependent on the host for soluble carbohydrates for their carbon source (Hayman, 1983). Evidence is not readily available on the competition between the mycorrhizal symbiont and plant pathogens. However, it is quite possible that such pathogens as the endoparasitic nematodes, which require nutrients from the host for reproduction and development would compete for the same pool of host photosynthate as the VA mycorrhizal fungi (Graham, 1988).

Direct competition between the mycorrhizal symbionts and soil-borne nematodes and fungi for space in or on root tissue has been postulated as a mechanism of inhibition of pathogens (Dehne, 1982; Graham, 1988). Presence of nematodes in roots was shown to be inhibited by the presence of VA mycorrhizal fungal structures (Saleh and Sikora, 1984; Grandison and Cooper, 1986; Smith *et al.*, 1986b). Davis and Menge (1980) also suggested that *P. parasitica* and *G. fasciculatum* competed for space on citrus roots. By the same token, larger root systems, attributed to VA mycorrhizal fungi, may provide more sites of infection available to pathogenic nematodes and fungi (Hussey and Roncadori, 1977; Francl and Dropkin, 1985). It is often difficult, though, to assess when the pathogen or VA mycorrhizal fungus inhabit the root site and which organism becomes established first in the root. Further detailed studies are required to explore this problem.

3. Physiological and biochemical alterations of the host.

Many researchers believe that physiological and biochemical mechanisms, other than improved phosphorus nutrition, are responsible for the biological control of soil-borne pathogens by VA mycorrhizal fungi. Baltruschat and Schönbeck (1975) observed higher levels of arginine in mycorrhizal plants than nonmycorrhizal plants which increased tobacco resistance to *T. basicola*. Increases in arginine were associated with increased activity of enzymes involved in the ornithine cycle (Dehne *et al.*, 1978). The ornithine cycle is stimulated in mycorrhizal roots and is associated with the formation of arbuscules. Roots which

are colonized by VA mycorrhizal fungi exhibited high chitinase activity where chitinase is believed to be involved in the degradation of the arbuscule by the host (Dehne *et al*., 1978). This increased activity of chitinase may be associated with degradation of chitin-rich cell walls of some soil-borne pathogens.

A localized influence of VA mycorrhizal fungi is the lignification of plant tissue, particularly in the stelar area (Dehne and Schönbeck, 1979b). Tolerance of mycorrhizal tomato plants to *F. oxysporum* was associated with increased lignin synthesis. The authors suggested that lignin deposition was brought about by phenol synthesis, through the increase in the lignin precursors, phenyl propanes. Krishna and Bagyaraj (1983) observed an inhibition of growth of *Sclerotium rolfsii* Sacc. by ortho-dihydroxy (O-D) phenols at concentrations comparable to those found in peanut plants infected with *G. fasciculatum*. Two amino acids, phenylalanine and serine, were found in higher concentrations in roots inoculated with VA mycorrhizal fungi and were observed to have nematicidal properties (Suresh and Bagyaraj, 1984).

In soybean, three isoflavonoids accumulated in mycorrhizal roots at levels lower than 1 μg per gram root (Morandi *et al*., 1984). It was postulated that mycorrhizal roots accumulating isoflavonoids (which exhibit antimicrobial properties) may be more resistant to plant pathogenic fungi and nematodes than nonmycorrhizal plants. Duchesne *et al*. (1987) suggested that production of antimicrobial compounds, such as phytoalexins, in ectomycorrhizal plant roots is correlated with increased resistance of plants to root pathogens. Further research is warranted to investigate the role of these phytoalexins in suppression of disease mediated by VA mycorrhizal fungi.

F. Conditions for success of VA mycorrhizal fungi as biological control agents

Despite the amount of research conducted on VA mycorrhizal fungi and their influence on soil-borne fungi and nematodes, our understanding of the interactions among the host-symbiont-pathogen is incomplete. To continue our evaluation of VA mycorrhizal fungi as potential biological control agents, we must consider that each system is complex and results can vary depending on the system being studied.

Soil factors such as temperature, pH, salinity, and fertility levels can differ among studies (Schenck and Kellam, 1978; Caron, 1989). Research on

mycorrhizal fungus-pathogen interactions should be conducted under several phosphorus regimes in order to avoid confounding tolerance or resistance of mycorrhizal plants to improved phosphorus nutrition alone (Graham, 1988). Differences in substrates can also influence the interaction between the pathogen and mycorrhizal symbiont (Caron *et al.*, 1985). It was suggested that one type of soil medium be used as a standard for comparison of interaction studies. However, one must be careful with interpretation of results if studies are to be conducted in soilless media, such as Turface (or calcined montmorrillonite clay), as these results may reflect artificial conditions.

Consideration should be given to the aggressiveness and inoculum level of the pathogen used. The level of disease as a result of pathogen inoculum should not produce symptoms so severe as to preclude any beneficial effects which may result through root colonization or improvement in growth by VA mycorrhizal fungi (Kellam and Schenck, 1978; Graham, 1988). The sequence of pathogen inoculation in relation to VA mycorrhizal inoculation may affect the ability of the VA mycorrhizal fungus to reduce disease severity (Hussey and Roncadori, 1982; Caron *et al.*, 1986c). However, preinoculation of plants with VA mycorrhizal fungi may represent an artificial system for crops which are seeded directly into fields as opposed to crops which are transplanted (Graham, 1988).

The susceptibility or resistance of the host to the pathogen may affect the ability of the VA mycorrhizal fungus to control disease (Cooper and Grandison, 1986; Grandison and Cooper, 1986). Furthermore, the reaction of the host may differ according to the species or strain of VA mycorrhizal fungus utilized. One VA mycorrhizal species may confer more resistance to disease than another species (Davis and Menge, 1981). It has been suggested that a cocktail mix of several species of mycorrhizal fungi be utilized for the plants to ensure that a maximum benefit is achieved (Cooper and Grandison, 1987). Consideration should also be given to using VA mycorrhizal fungi indigenous to soils in the geographical area being studied. Indigenous species may be collected, pot cultured and later re-introduced into field soils in greater spore densities and strategically placed to ensure that plant roots will encounter the inoculum. The indigenous species may also be able to tolerate soil conditions unique to specific soils.

More information is needed to determine the amount of time required for suppression of disease by the VA mycorrhizal fungus to occur (Caron, 1989). In addition, few studies have reported on the stability of the interaction between pathogen and mycorrhizal symbiont over time (Caron *et al.*, 1986a). The level of

mycorrhizal inoculum necessary to achieve biological control as well as placement of inoculum should be given more consideration. Generally, 0.5 to 5.0 spores of VA mycorrhizal fungi per gram of soil have been used to produce plant growth responses (Graham, 1988). Caron (1989) stressed the need for comprehensive research on the mechanism(s) involved in disease control by VA mycorrhizal fungi. Once a better understanding on the mechanisms involved in disease suppression is achieved, we may acquire some knowledge regarding the manipulation or modification of the system to improve the performance of VA mycorrhizal fungi as biological control agents.

G. Objectives of the thesis

The objectives of the thesis were to study the VA mycorrhizal fungi associated with barley and other field crops and to investigate the ability of VA mycorrhizal fungi to control barley common root rot disease caused by *Bipolaris sorokiniana* . The objectives are outlined as follows:

1. To collect and identify species of VA mycorrhizal fungi indigenous to some agricultural soils in Alberta and to use SEM and X-ray microanalysis to determine the levels of calcium in spore walls of the VA mycorrhizal fungi.
2. To study the susceptibility of barley cultivars to VA mycorrhizal fungi.
3. To study the interaction between VA mycorrhizal fungi and *B. sorokiniana* .
4. To investigate possible mechanism(s) by which VA mycorrhizal fungi may reduce the severity of barley common root rot disease. The role of phosphorus, phytoalexins, and root exudates in the suppression of disease by VA mycorrhizal fungi will be examined.

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Chapter II

Vesicular-Arbuscular Mycorrhizal Fungi Associated with Some Field Crops in Alberta

A. Introduction

Interest in vesicular-arbuscular (VA) mycorrhizal fungi in Canada has increased tremendously over the last decade. Taxonomic identification is a must for isolate typification before any meaningful experimental work can be undertaken. The American Phytopathological Society identified this area as a research priority two years ago. Although the number of species of VA mycorrhizal fungi discovered world-wide is increasing, very little effort has been initiated to document those species indigenous to Canadian soils. Six species of *Glomus* Tul. & Tul. from Québec were first described by Thaxter (1922). Other collections of VA mycorrhizal fungi from eastern Canada have been reported (Furlan and Fortin, 1972; Herskowitz and Estey, 1978; Berch and Kendrick, 1982; Berch and Fortin, 1983, 1984). A limited number of studies reporting the geographic distribution of the Endogonaceae have been conducted in the western Canadian provinces of British Columbia (Gerdemann and Trappe, 1974; Molina *et al.*, 1978; Cade-Menun *et al.*, 1991), Alberta (Zak *et al.*, 1982; Zak and Parkinson, 1983; Danielson *et al.*, 1984; Boyetchko and Tewari, 1986), and Saskatchewan (Kucey and Paul, 1983). Zak *et al.* (1982) reported the presence of *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe, *G. aggregatum* Schenck & Smith emend. Koske, *G. tenue* (Greenall) Hall, and *Entrophospora infrequens* (Hall) Ames & Schneider in amended mine spoils in Alberta. A new species, *G. dimorphicum* Boyetchko & Tewari, was reported to be associated with barley in Alberta (Boyetchko and Tewari, 1986).

Several reports have documented the association of VA mycorrhizal fungi with agricultural crops in western Canada (Kucey and Paul, 1983; Kucey and Diab, 1984; Zak and Parkinson, 1983; Boyetchko and Tewari, 1986; Hwang, 1988; Cade-Menun *et al.*, 1991). However, authors do not always specify which VA mycorrhizal species are used in each study (Kucey and Diab, 1983; Hwang, 1988). Since some hosts vary in their VA mycorrhizal root colonization (Boyetchko and Tewari, 1990), it is important to be cognisant of the particular VA mycorrhizal species being used in an experiment. Therefore, if further research, particularly field trials, on VA mycorrhizal fungi is to be continued in western Canada and

results compared with other reports, further collections and identification of species indigenous to the region is necessary.

The purpose of this study was to collect and identify some species of VA mycorrhizal fungi associated with field crops in Alberta. Although this was not an exhaustive taxonomic/ecological study, the information presented extends the known geographic distribution of five VA mycorrhizal species. This study also provides some new information on the chemical and physical characteristics of the VA mycorrhizal fungal spore walls. Special emphasis was placed on studying the relative levels of calcium in different cell wall layers as this aspect has been reported for *G. dimorphicum* (Boyetchko and Tewari, 1986) but has generally received little attention in fungi. The species collected in this study have been pot cultured and will be available as ecotypes of VA mycorrhizal fungi for further experimentation.

B. Materials and Methods

Soil samples were collected in 1986 and 1987 during the month of July from various sites in Alberta where barley, red clover, and alfalfa were grown, brought back to the laboratory, and stored for one to two weeks at 4 C until processing. Most spores directly isolated from the field soils were either parasitized or some walls of the spores were indistinguishable. Therefore, all VA mycorrhizal fungi were pot cultured before identifying them to species and single spore isolates of each species were obtained. The VA mycorrhizal fungi obtained from these soils were multiplied in pot culture by placing approximately 250 g of the field soil below the seed of alfalfa (*Medicago sativum* cv. Beaver) in 15 cm pots containing a potting medium consisting of steam sterilized sand and soil (3:1). The pots were fertilized with full strength Hoagland's solution, minus phosphorus and adjusted to pH 6.0 once a week, in addition to regular watering. Greenhouse temperatures were 25-27 C during the day and maintained at 20 C during the night. The photoperiod was 16 hours with a maximum light intensity of 500 $\mu E/m^2/s$. Pot cultures were harvested after 8 to 10 months.

Spores were collected by wet-sieving and decanting of soil according to the method of Gerdemann and Nicolson (1963). Approximately 100 g soil was placed in a Waring blender with 250 ml water for approximately 10 to 20 seconds, passed through a series of sieves (sizes 710, 425, 250, 150, 106, and 45 μm) on a sieve shaker, and water allowed to wash through each sieve for 30 minutes (Fig. II-1). The material retained on the sieves was washed and collected onto Whatman 1 filter

paper under suction using a Millipore apparatus (Fig. II-2). In some cases, a sucrose gradient was employed to collect the VA mycorrhizal spores (Dr. J. Morton, personal communication to Dr. J.P. Tewari). The sucrose gradient was prepared by pipetting 15 ml of 60% sucrose below 15 ml of 20% sucrose in a 50 ml centrifuge tube. Spores retained on a sieve as previously described were washed from the sieve, placed in solution, pipetted onto the sucrose gradient, and spun at 3000 rpm in a desk-top centrifuge for 5 minutes. The spores suspended at the sucrose interface were collected with a pipette, washed several times with water on a 45 µm sieve to remove the sucrose, and collected onto filter paper as previously described.

VA mycorrhizal fungal spores were examined under a stereoscopic microscope and picked up, individually, with a dissecting needle. Spores were observed by light microscopy by mounting them in either lactophenol (1 part phenol, 1 part lactic acid, 1 part glycerol, and 1 part water, by weight) or in a polyvinyl alcohol solution (PVA) (1.66 g polyvinyl alcohol, 10.0 ml water, 10.0 ml lactic acid, and 1.0 ml glycerol). Some spores were mounted in Melzer's reagent, which reacts to components of the wall and provides information on the extent of polymerization of the cell wall components. A blue color, or amyloid reaction, will indicate the presence of starch, whereas a red color, or dextrinoid reaction, will indicate the presence of high concentrations of quaternary ammonium compounds (Morton, 1988).

Spores prepared for scanning electron microscopy (SEM) were placed onto filter paper, air-dried, and vapor-fixed with 2% osmium tetroxide in water overnight. Spores were then mounted onto stubs, coated with gold, and examined in a Cambridge Stereoscan 150 SEM. In some cases, VA mycorrhizal fungal spores were fractured with a dissecting needle to obtain cross-sectional views of the spore walls. Energy dispersive X-ray microanalyses were carried out in the same instrument using a Kevex Micro-X 7000 analytical spectrometer. In this method, atoms are excited by a high-energy electron beam, and X-rays of a certain wavelength and energy are emitted which are characteristic for a particular element (Brown and White, 1982). When an electron beam is held in one spot of a sample, the X-rays emitted from that site can be analyzed. By this technique, elements between sodium and uranium in atomic weight can be detected (Goldstein *et al.*, 1981). The angle of incidence of the electron beam is normalized and the zone of analysis for detection of an element in the sample is approximately 1-2 µm. Information provided on the X-ray spectra includes the VFS which refers to

vertical full scale (i.e. the number of counts on the graph to the maximum point on the graph), and the number, 10.240, which refers to the energy (in volts) of the X-rays. Semi-quantitative comparisons of elements in the sample can be made by using the K-ratio, which is normalized within each sample. The K-ratio refers to X-ray emissions, from the K electron orbit, which are collected and quantified. Gold is used as a standard and other elements compared to it.

The studies of spore morphology and X-ray microanalysis of the spore cell wall were also carried out on the spores of *G. intraradices* Schenck & Smith and *G. mosseae*, which were used extensively in the various experiments in this research. These species were originally obtained from the International Culture Collection of VA Mycorrhizal Fungi (INVAM) at the University of Florida, Gainesville from Dr. N.C. Schenck. The cultures were maintained in the same manner as described earlier. For comparison, X-ray microanalyses were also done on the cell walls of spores and hyphae of *Bipolaris sorokiniana* (Sacc.) Shoem. and on barley roots and root hairs. Cultures of *B. sorokiniana* were grown on potato-dextrose agar (PDA) for ten days. The spores and hyphae of *B. sorokiniana*, and barley roots were prepared for SEM in the same manner as the spores of VA mycorrhizal fungi.

C. Results

Five VA mycorrhizal species were identified from agricultural soils. For each species, a minimum of 50 spores were examined to determine spore sizes and spore wall thickness. The following provides information on the collection site, host plant with which these fungi were associated, and some comments on the morphology of each species. In addition, some new information on the VA mycorrhizal species collected, including elemental analysis of spore walls by X-ray microanalysis, is presented. Information is also summarized from data collected for *G. intraradices* and *G. mosseae* , *B. sorokiniana* spores and hyphae, and barley roots and root hairs.

1. *Glomus aggregatum* Schenck & Smith emend. Koske

The spores of *G. aggregatum* were isolated from soil and roots associated with alfalfa (*Medicago sativum*) in Devon (Lat. 53º 22' N, Long. 113º 44' W). The spores formed in loose clusters (sporocarps) with as many as 100 or more

spores per cluster (Figs. II-3, II-4, II-5). Spores were globose, ranging in size from 50-80 µm in diameter and the spore wall morphology was similar to that described for *G. aggregatum* by Koske (1985). Mature spores were pale yellow to yellow brown and had outer hyaline, middle laminate and thin membranous walls (Fig. II-3). The spores of this isolate consistently developed the outer, hyaline wall, which is known to occur occasionally in *G. aggregatum* (Koske, 1985). The laminate wall was 1-3 µm thick and the membranous wall was approximately 0.5 µm thick. The inner membranous wall had a greenish tint when viewed by light microscopy (Fig. II-3b). External proliferation into secondary spores was observed from some spores (Fig. II-3b, II-4). This was reminiscent of precocious or microcyclic sporulation in some fungi (Allermann *et al.*, 1983; Pezet *et al.*, 1983; Kristiansen and Al-Rawi, 1986) and is the first report of external proliferation in *G. aggregatum*. X-ray microanalysis revealed a concentration of calcium in the middle wall (Fig. II-6b, Table II-1). The outer hyaline wall had relatively less calcium than the middle wall (Fig. II-6a, Table II-1).

2. *Glomus pansihalos* Berch & Koske

The spores of *G. pansihalos* were collected from the same soil sample as *G. aggregatum* in the Devon area. The spores have been observed mostly as single spores but occasionally formed groups of 2 to 5 spores. Color of spores was yellow brown to dark brown and were usually subglobose, ranging in size from 150-180 x 120-160 µm. The spores developed three walls: an outermost wall which was 3-6 µm thick and expanded in PVA (Figs. II-7, II-8), a middle laminate wall, 3-6 µm thick, whose outer surface was composed of hemispherical warts (Fig. II-9), and a thin, inner unit wall which was 1-2 µm thick (Figs. II-7, II-8). The sporogenous hypha was cylindrical and composed of 1-3 walls. X-ray microanalysis showed the presence of calcium in the outer expanding wall and the middle wall (Fig. II-10, Table II-1). However, the middle, laminate wall had relatively higher levels of calcium than the outer wall (Fig. II-10b, Table II-1).

3. *Glomus tenue* (Greenall) Hall

Glomus tenue was collected from soil in which barley was grown for six years at the University of Alberta Research Farm in Edmonton (Lat. 53° 33' N, Long. 113° 40' W). Roots of barley from the field and barley grown in the

greenhouse in soil containing these fungi contained some fine hyphae, in addition to small spores, in the rhizosphere (Fig. II-11). The fan-shaped structures in the roots described by Hall (1977) were not observed here. The spores were 8 to 20 µm in diameter and stained blue with lactophenol cotton blue (Fig. II-11). The spores were too small to be readily isolated from the soil and, therefore, were not examined by SEM or X-ray microanalysis.

4. *Entrophospora infrequens* (Hall) Ames & Schneider

The spores of *E. infrequens* were associated with red clover roots at Breton (Lat. 53° 10'N, Long. 114° 30' W). Attempts to establish pure pot cultures of this species have failed, as was the case with Ames and Schneider (1979). However, *E. infrequens* occurred as a common contaminant in pot cultures of *G. dimorphicum*. The spores of *E. infrequens* develop from a vesicle (or sporiferous saccule) which empties as the spore develops (Figs. II-12, II-13a). Spores were globose to subglobose, ranging in size from 110-130 x 100-130 µm while the vesicle was globose to subglobose ranging in size from 110-130 x 110-130 µm. The spores were enclosed by the hyaline wall of the vesicular stalk (Figs. II-12, II-13c) and possessed an ornamented wall, 2-8 µm thick, consisting of vacuolated spines (Figs. II-12, II-13b, II-13c, II-13d). X-ray microanalysis revealed a concentration of calcium in the wall of the vesicle as well as the ornamented wall (Fig. II-14, Table II-1). The amount of calcium present in both walls was found to be similar (Fig. II-14, Table II-1).

5. *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe

The spores of *G. mosseae* were isolated from barley fields at the Lacombe Research Station (Lat. 52° 28' N, Long. 113° 45' W), as well as from a red clover field in Fairview (Lat. 56° 10' N, Long. 118° 25' W). The spores were mostly formed singly in soil and were yellow to brown in color. Spores ranged in size from 140-300 µm in diameter, and possessed 1 wall which was 1-4 µm thick (Fig. II-15). The sporogenous hypha was generally funnel-shaped to cylindrical (Figs. II-18a, II-18b) with a curved septum separating the spore contents from the hypha (Fig. II-15b). X-ray microanalysis showed relatively low levels of calcium in the wall (Fig. II-19a, Table II-1).

The spores of *G. mosseae* originally obtained from INVAM formed singly in soil as well as in sporocarps and resembled those spores collected from the Alberta soils (Fig. II-16). No hyphal mantle surrounding the spores was observed. Analysis of the spore wall for calcium revealed similar calcium levels as those obtained from the Alberta isolates (Figs. II-19b, Table II-1).

6. *Glomus intraradices* Schenck & Smith

Spores of *G. intraradices* formed as individual spores and in spore clusters in the roots as well as in the soil (Figs. II-17, II-18c). Although it has been described by other authors that this species forms spores rarely outside the root (Schenck and Smith, 1982), sporulation of this species in pot culture, using alfalfa as host, occurred very often in the soil, close to the roots. The spore walls were complex, with several laminate wall layers forming in larger, mature spores (Fig. II-17a). An outer hyaline, ephemeral wall layer was often observed (Fig. II-17a). Analysis of the outer wall by X-ray microanalysis revealed a concentration of calcium in the wall (Fig. II-20a). Because of the complexity of the spore wall layers of *G. intraradices*, separation of the wall layers for SEM was difficult. Therefore, analysis for calcium was made on the outer wall layer, in addition to the inner-most wall layer by observing spores from the inside (Fig. II-18d). The inner-most wall layer contained relatively higher levels of calcium than the outer wall layer (Fig. II-20b, Table II-1). X-ray microanalysis of soil particles agglutinating to the spore wall of *G. intraradices* revealed the presence of silicon, aluminum, iron, and potassium, in addition to calcium (Fig. II-21). The surface of other VA mycorrhizal spores had soil particles adhering to their walls. Other microanalyses showed similar results as those seen with the soil particles on *G. intraradices* spores.

7. *Bipolaris sorokiniana* (Sacc.) Shoem.

Cultures of *B. sorokiniana* were grown on potato-dextrose agar for 10 days and spores and hyphae were examined (Figs. II-22a, II-22b) for calcium in order to compare calcium levels in cell walls of this fungus with those of the VA mycorrhizal fungi. X-ray microanalyses of both the spore and hyphae revealed relatively low levels of calcium when compared to spore walls of VA mycorrhizal

fungi in general (Fig. II-23, Table II-1). Potassium was observed in the walls of both spores and hyphae (Fig. II-23).

8. Barley (*Hordeum vulgare* L.) roots and root hairs

The roots (Fig. II-22c) contained relatively low levels of calcium as revealed by X-ray microanalysis (Fig. II-24, Table II-1). However, root hairs (Fig. II-22d) had higher levels of calcium than the roots, but much lower levels of calcium when compared to some of the spore walls of the VA mycorrhizal fungi examined (Fig. II-24, Table II-1). Potassium was observed in both the roots and root hairs, with higher levels of potassium occurring in the root hairs (Fig. II-24).

D. Discussion

Five species of VA mycorrhizal fungi were isolated from agricultural soils in Alberta. *Glomus mosseae* was isolated from two sites, associated with barley and red clover at Lacombe and Fairview, respectively. Zak *et al.* (1982) reported that *G. mosseae* was one of the most common VA mycorrhizal species isolated from mine spoils located near Fort McMurray. *Glomus aggregatum* was also isolated from soil near Devon, and has been collected from peat deposits near Camrose (Danielson *et al.*, 1984) and from mine spoils near Fort McMurray (Zak *et al.*, 1982). From these reports combined, it appears that both *G. mosseae* and *G. aggregatum* have been the most frequently encountered VA mycorrhizal species in Alberta to date.

Definitive identification of *G. aggregatum* was difficult because these spores are similar to those of *G. fasciculatum* (Thaxter) Gerd. & Trappe emend. Walker and Koske. Detailed examination of the spores and comparison with emended descriptions of *G. aggregatum* (Koske, 1985) and *G. fasciculatum* (Walker and Koske, 1987) indicated that the spores matched those described for *G. aggregatum*. The outer, hyaline wall seen in some spores of *G. aggregatum* was also observed in another Alberta isolate by Zak *et al.* (1982). The innermost wall appears to be similar to that described by Walker (1983). This wall had a greenish color when viewed by bright-field illumination and, thus, matches the description by Koske (1985). Failure of the innermost wall to stain dark red-brown with Melzer's reagent, as seen with *G. fasciculatum* (Walker and Koske, 1987) confirmed that this species was *G. aggregatum* (Koske, 1985). Internal

proliferation of spores of *G. aggregatum* has been reported (Koske, 1985). Spores of this species isolated from Alberta sometimes showed external proliferation of a spore from another spore, with the thin, hyaline, outer wall forming around the spore and continuing around the hypha. This type of sporulation has not been described for *G. aggregatum* until now. This phenomenon is characterized by the production of second-generation spores connected to the parent spores by shortened germ tubes (Allermann *et al.*, 1983). Temperature was a factor involved in microcyclic sporulation of *Aspergillus niger* Van Tiegham spores while elemental sulfur induced this condition in spores of *Phomopsis viticola* Sacc. Microcyclic sporulation eliminates the vegetative growth stage and results in greater spore production.

Entrophospora infrequens was also recovered from field soil, and has been reported from mine spoils in Alberta (Zak *et al.*, 1982). Although successful pure pot cultures of this species could not be established, it did occur readily as a contaminant in some pot cultures of *G. dimorphicum*, which are being maintained in the Department of Plant Science greenhouses. Contamination of *G. mosseae* pot cultures with *E. infrequens* has also been reported (Ames and Schneider, 1979). It was noted that *E. infrequens* did not parasitize the spores of *G. mosseae*, and this has also been the case in our study.

The fine endophyte, *G. tenue*, was observed in roots of barley growing at the University of Alberta research farm in Edmonton. This species was also found in slender wheatgrass roots which were grown in peat-amended mine spoils (Zak *et al.*, 1982). Since *G. tenue* was only observed in plant roots grown in the peat-amended soil, the authors believed that *G. tenue* was introduced with the peat, collected from a spruce forest. The location of the spruce forest from which the peat moss was obtained was not provided in the paper. Sporulation of *G. tenue*, although limited, was observed in the barley roots. *Glomus tenue* has been largely recognized by the morphology of infection within the root (Hall, 1977). However, barley grown in the field at the University of Alberta farm contained low levels of VA mycorrhizal colonization (Boyetchko, 1986; see Chapter III) and another as yet unidentified *Glomus* species was present in barley roots grown in the field. It was, therefore, difficult to determine exact amounts of barley roots colonized by *G. tenue*.

The isolation of spores of *G. pansihalos* from Devon is the first report of this species from Alberta. This species was readily recognized by the outer, hyaline wall which expands in the presence of lactic acid. By lowering the pH of the

mounting medium, lactic acid elicits the expanding wall. An additional feature of *G. pansihalos* which was observed was the middle, laminate wall whose surface was covered with hemispherical warts (Berch and Koske, 1986). *Glomus pansihalos* has been reported from southern Ontario soils, associated with fern roots (Berch, 1979), as well as from sand dune soils in California, New Jersey, and Michigan (Berch and Koske, 1986).

Energy dispersive X-ray microanalysis was a technique employed to study the characteristics of the spore walls of VA mycorrhizal fungi isolated from the field soil. This method has been previously used by Boyetchko and Tewari (1986) to examine the outer and middle wall layers of *G. dimorphicum* spores. Examination of the VA mycorrhizal spore walls revealed that the spore walls of VA mycorrhizal fungi contain relatively high levels of calcium. Semi-quantitative analysis showed that, for some species, one wall may contain more calcium than another wall, for the same spore. The outer hyaline wall of *G. aggregatum* had lower amounts of calcium than the middle wall. Similarly, the ornamented wall of *G. pansihalos* spores contained more calcium than the outer wall and examination of the innermost wall of *G. intraradices* revealed a greater concentration of calcium than the outer wall. In addition, it has been reported that the outer evanescent wall of *G. dimorphicum* had higher levels of calcium than the middle laminate wall (Boyetchko and Tewari, 1986). No distinct differences in calcium levels of spore walls among VA mycorrhizal species examined were observed. However, comparison with another non-mycorrhizal fungus, *B. sorokiniana*, showed that calcium levels in the wall of the spores and hyphae were relatively low. The reason for the relatively high levels of calcium in spore walls of VA mycorrhizal fungi and not in *B. sorokiniana* is not clear. It is possible that VA mycorrhizal fungi have inherently higher levels of calcium in their walls. Since *B. sorokiniana* was grown on PDA, while the VA mycorrhizal fungi were collected in soil, the culture media may have some effect on the chemical nature of spore walls of *B. sorokiniana*. In higher plants, calcium binds to pectins present in the middle lamella (Demarty *et al.*, 1984). Some calcium was observed in roots and root hairs of barley, although not in relatively high amounts. Since the zone of penetration of the electron beam is 1-2 μm, calcium could not be detected in the plant roots below 1-2 μm where the greatest concentration of calcium, in the form of calcium pectate, occurs.

The importance of calcium in the cell walls of VA mycorrhizal fungi is not clear. Calcium oxalate crystals have been reported in the walls of fungi belonging

to the Mucorales (Jones *et al.*, 1976; Urbanus *et al.*, 1978; Whitney and Arnott, 1986). Crystals were observed to be firmly embedded in the cell walls of zygophores and sporangiophores of *Mucor mucedo* Brefeld (Urbanus *et al.*, 1978). Pitt and Ugalde (1984) speculated that calcium oxalate crystals in fungal cell walls may be an end-product of fungal metabolism and may, therefore, be secreted through the plasma membrane. Whitney and Arnott (1986) suggested that the crystals may detoxify calcium ions in fungi. They also proposed that the random, interlocking network of calcium oxalate crystals found in the sporangiophore wall of *Gilbertella persicaria* (Eddy) Hesseltine may provide rigidity to the fungal wall. In lower fungi, such as *Phytophthora cactorum*, oospore production decreases under calcium-deficient conditions (Elliot, 1972). Fletcher (1979) similarly showed that low concentrations of calcium in growth medium resulted in high incidence of oogonium and oosphere abortion of *Saprolegnia diclina*. It is possible that the presence of calcium in the spore walls of VA mycorrhizal fungi signifies the importance of this element in growth and sporulation. High levels of calcium are also known to induce resistance against many pathogens (Kunoh *et al.*, 1986; Vidhyasekaran, 1988). Formation of papillae, a thickening of host cell wall, represents a dynamic host response to pathogens. Kunoh *et al.* (1986) observed that papillae in barley coleoptiles, formed in response to infection by *Erysiphe graminis*, contained appreciable levels of calcium. Papillae have been reported in parasitized spores of VA mycorrhizal fungi (Boyetchko and Tewari, 1991). It is possible that these structures may contain high levels of calcium which may play a role in inducing resistance of mycorrhizal spores against parasites occurring in the soil. Inclusion of such information warrants further investigation.

The collection of VA mycorrhizal fungi from agricultural soils in this study, along with a previous collection of *G. dimorphicum* from a barley field at Neerlandia (Boyetchko and Tewari, 1986) indicates that these fungi are normal components of field soil. Their presence may significantly affect growth and yield of economically important field crops. Suppression of soil-borne diseases may be the result of root colonization by VA mycorrhizal fungi. Thompson and Wildermuth (1989) observed a negative correlation between severity of barley common root rot and VA mycorrhizal root colonization. Although agricultural soils are intensively managed through fertilizer and pesticide applications, as well as tillage practices, which result in soil disturbance, these fungi may have an ecological significance in the agricultural industry. Even though it has been reported that VA mycorrhizal spore densities are higher in undisturbed soils than in cultivated soils

(Kucey and Diab, 1983), the presence of VA mycorrhizal fungi in agricultural soils suggests that ecotypes, which tolerate site disturbance and high fertilizer and pesticide use, are present. Future work should consider, more extensively, the role of VA mycorrhizal fungi in agricultural cropping systems.

E. Table, Figures, and Legends

Table II-1. Comparison of Calcium Levels in Spore Walls by a Semi-Quantitative Analysis[a]

Specimen		Calcium (K-Ratio)[b]
Glomus aggregatum	Outer hyaline wall	0.257
	Laminate wall	0.624
Glomus pansihalos	Outer wall	0.377
	Ornamented wall	0.504
Entrophospora infrequens	Ornamented wall	0.517
	Vesicular envelope	0.543
Glomus mosseae (Alberta isolate)	Wall	0.363
Glomus mosseae (INVAM)	Wall	0.343
Glomus intraradices	Outer wall	0.679
	Innermost wall	0.802
Bipolaris sorokiniana	Spore wall	0.068
	Hyphal wall	0.015
Barley	Root	0.097
	Root hair	0.238

a. Semi-quantitative analysis was obtained by X-ray microanalysis. The normalized intensity ratio (K-ratio) was used for comparison between samples.

b. Calcium level in spore walls is the average of 10 observations.

Figure II-1. Sieve shaker and wet-sieving apparatus. Series of sieves ranging in size from 710 µm (top) to 45 µm (bottom). Note the rubber hose attached to the water tap on the laboratory bench (Fig. II-1b) which sprays a mist of water through a spray nozzle over the sieves. Another hose is attached to the bottom pan for drainage of water into the sink.

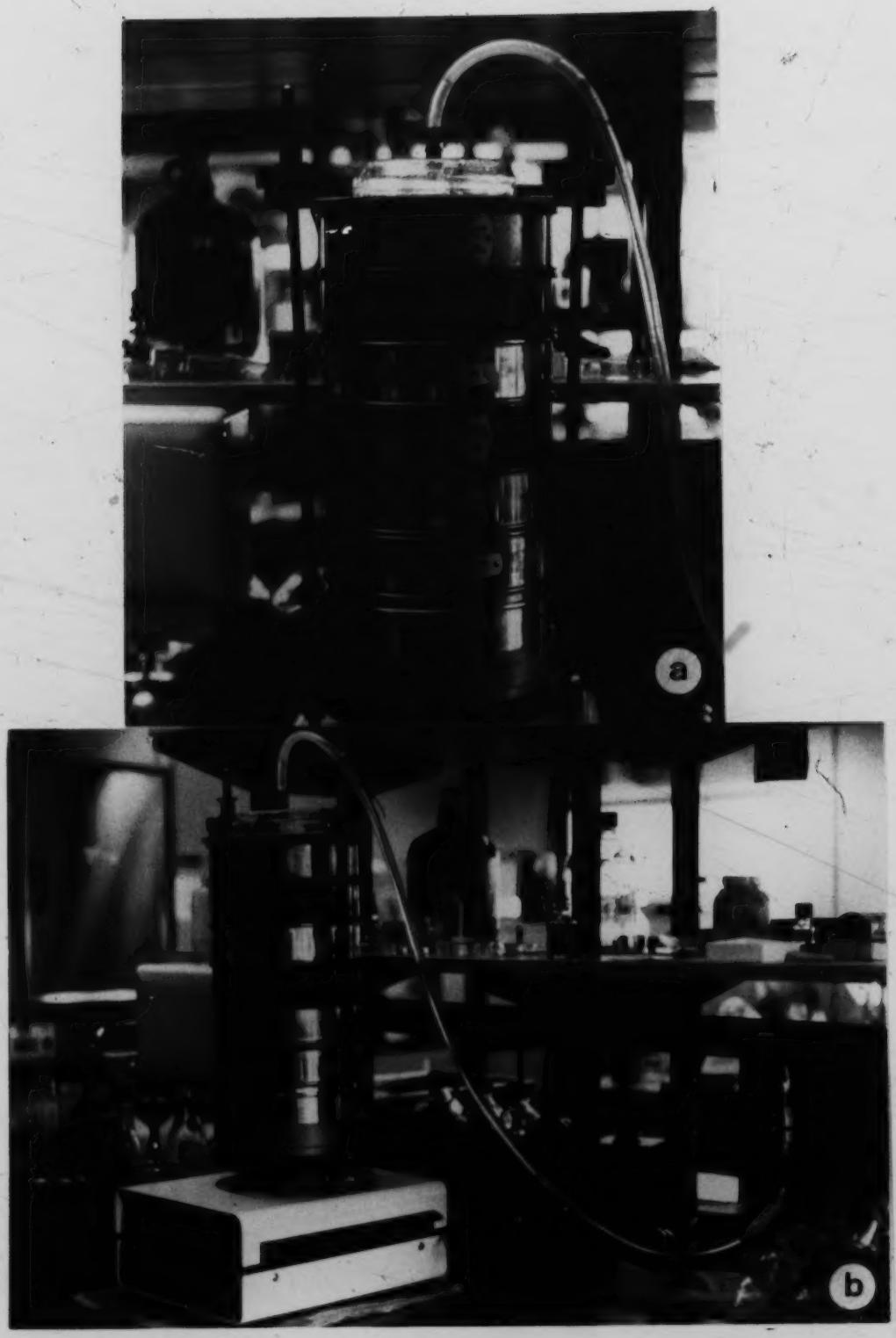
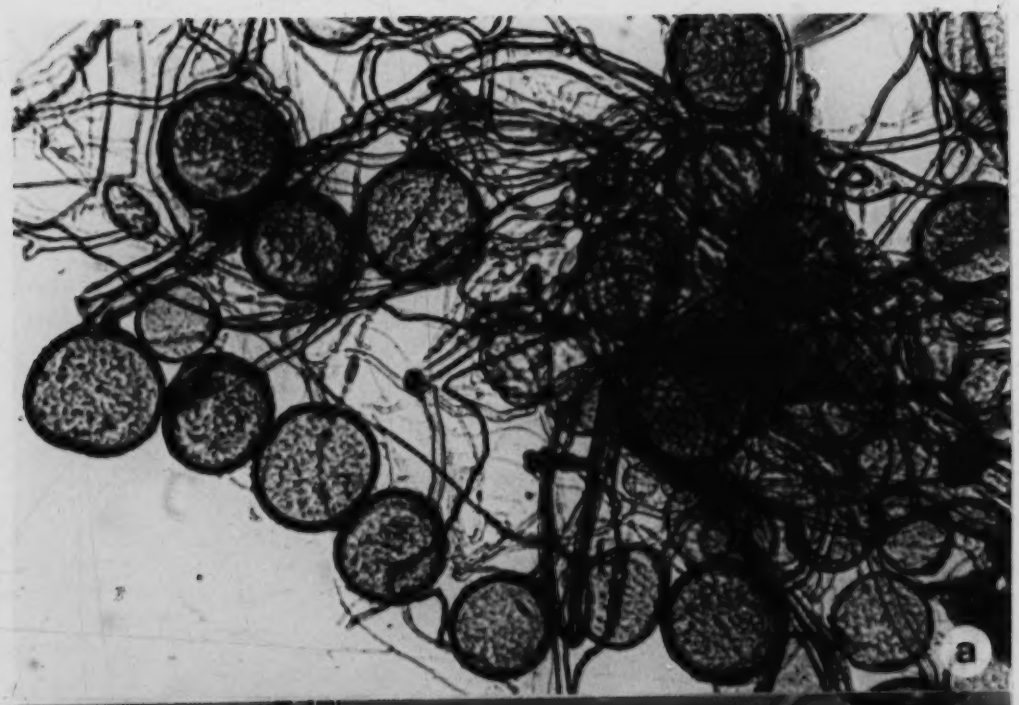

a
b

Figure II-2. Millipore apparatus for collection of VA mycorrhizal spores onto filter paper under suction.

a

Figure II-3. Light micrographs of *Glomus aggregatum* spores.
Fig. II-3a. Sporocarp containing many spores in a loose aggregate. X 200.
Fig. II-3b. External proliferation of spore. Note the thin, outer hyaline wall (arrowheads) which is continous around the hypha. The innermost wall has a greenish tint when viewed by bright-field illumination. X 515.


a

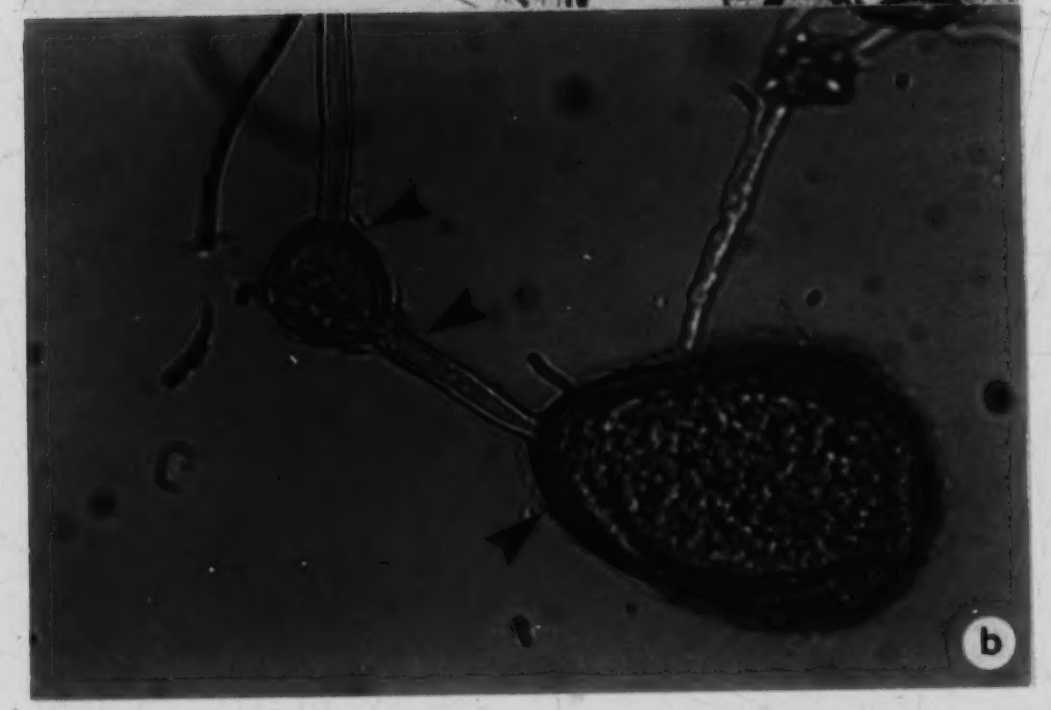

b

Figure II-4. Light micrographs of *Glomus aggregatum* spores.
Fig. II-4a. Spore showing outer hyaline wall (arrowhead). X 600.
Fig. II-4b. External proliferation of spore (arrowhead). Note that the growth of the hypha from the secondary spore ceases (arrow). X 600.

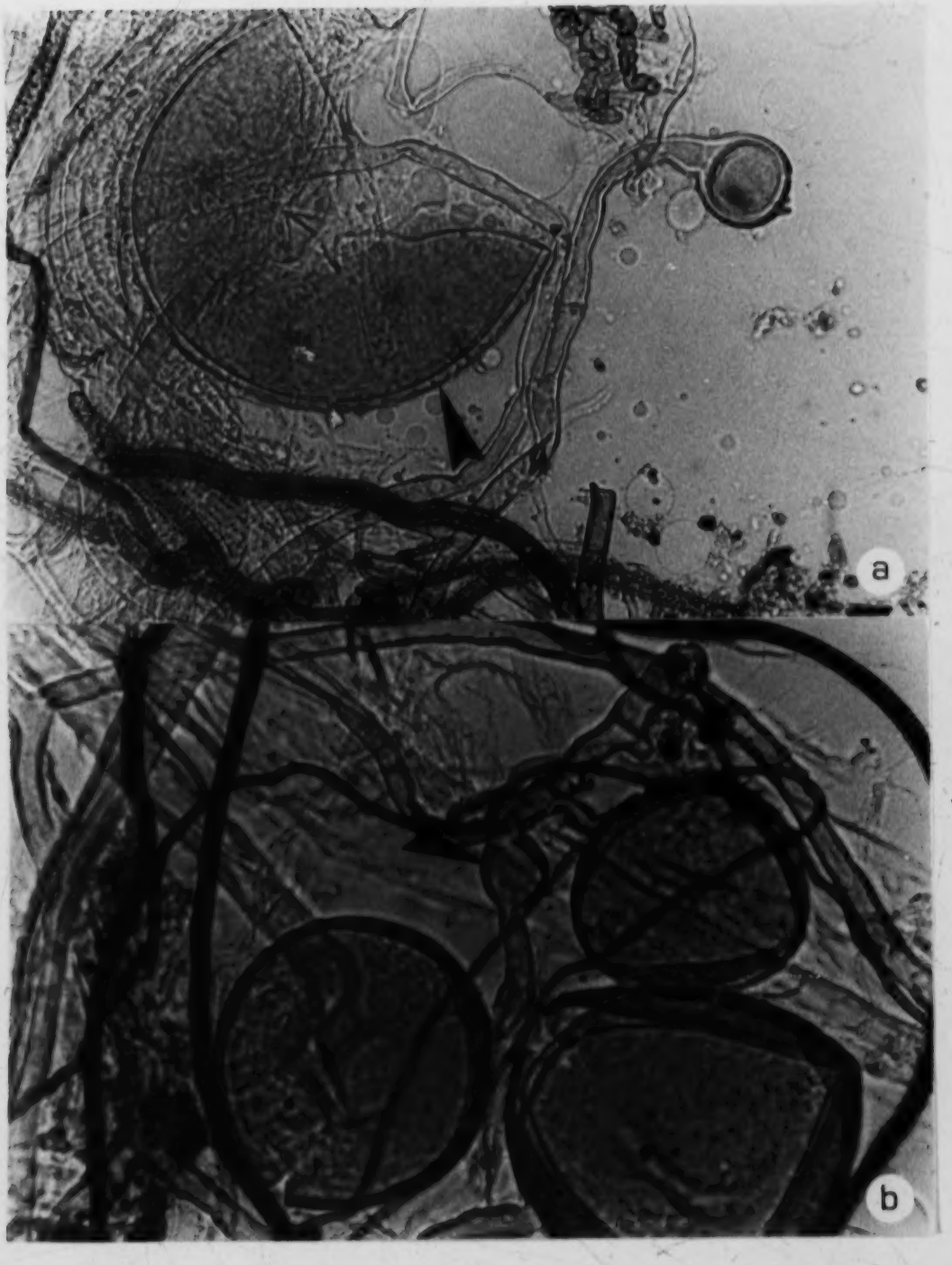

a
b

Figure II-5. SEM photographs of *Glomus aggregatum* spores.

Fig. II-5a, 5b. Sporocarp containing several globose to subglobose spores. II-5a X 40, II-5b X 150.

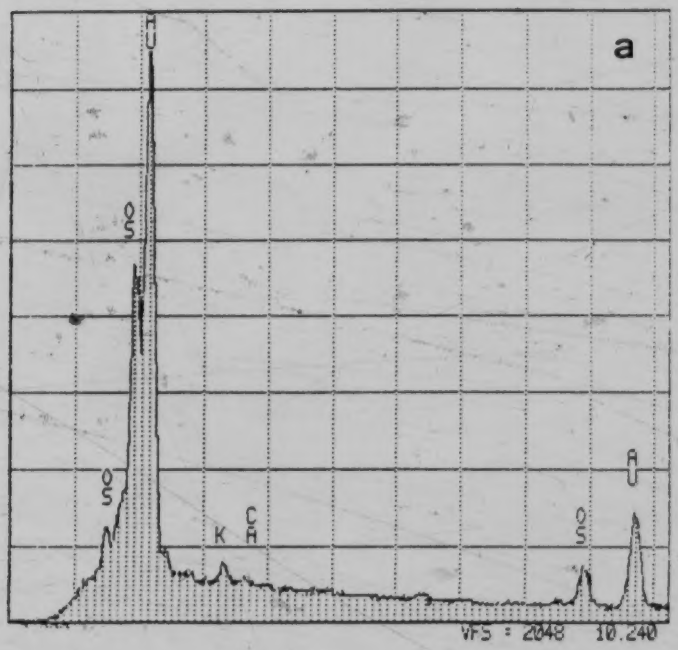
Fig. II-5c. Spore from the sporocarp. Various areas of the wall surface show the hyaline, outer wall which is roughened which may eventually slough-off (arrows) and the smooth surface of the middle wall (arrowheads). X 1200.

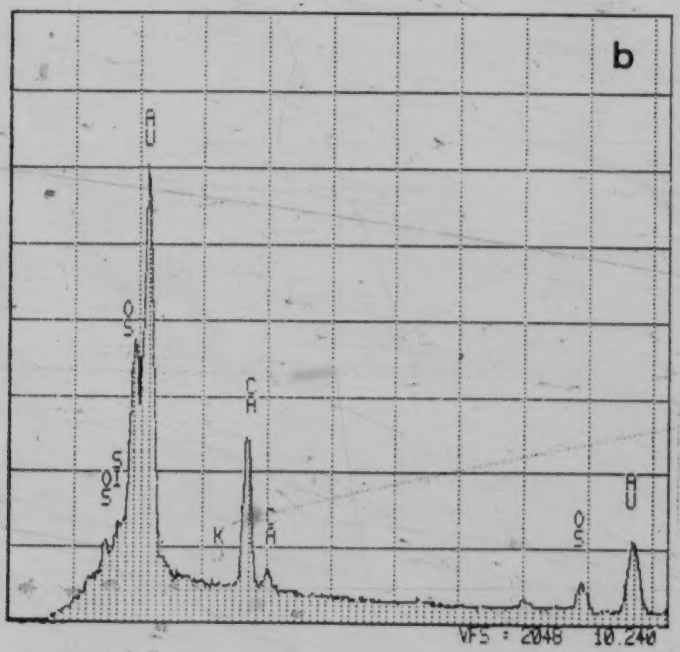
a
b
c

Figure II-6. X-ray spectra of spore walls of *Glomus aggregatum*.

Fig. II-6a. X-ray spectrum of the outer, hyaline wall. Note the low concentration of calcium in this wall.

Fig. II-6b. X-ray spectrum of second laminate wall revealed a greater amount of calcium than in the outer wall.


a
Au
O
S
O
S
K
Ca
O
S
Au
VFS = 2048 10.240


b
Au
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Ca
S
O
S
K
Ca
O
S
Au
VFS = 2048 10.240

Figure II-7. Light micrographs of *Glomus pansihalos* spores.
Fig. II-7a, 7b. Mature spores showing the outer wall which expands in the presence of lactic acid (large arrowheads), colored laminate wall (arrows) and colored unit wall (small arrowheads). II-7a X 515, II-7b X 759.

a
b

Figure II-8. Light micrographs of *Glomus pansihalos* spores.

Fig. II-8a. Mature spore showing outer expanding wall (large arrowhead), colored laminate wall (small arrow) and colored unit wall (small arrowhead). Note that the spore contains a filamentous parasite (*). X 730.

Fig. II-8b. Mature spore showing ornamention of laminate wall (arrowheads). X 730.

a
b

Figure II-9. SEM photographs of *Glomus pansihalos* spores and spore walls.

Fig. II-9a. Mature spore showing laminate wall covered with hemispherical warts (arrowhead). X 550.

Fig. II-9b. Enlarged view of outer expanding wall (arrow) and laminate wall covered with hemispherical warts (arrowhead). X 1200.

Fig. II-9c. Enlarged view showing hemispherical warts of the laminate wall. X 7000.

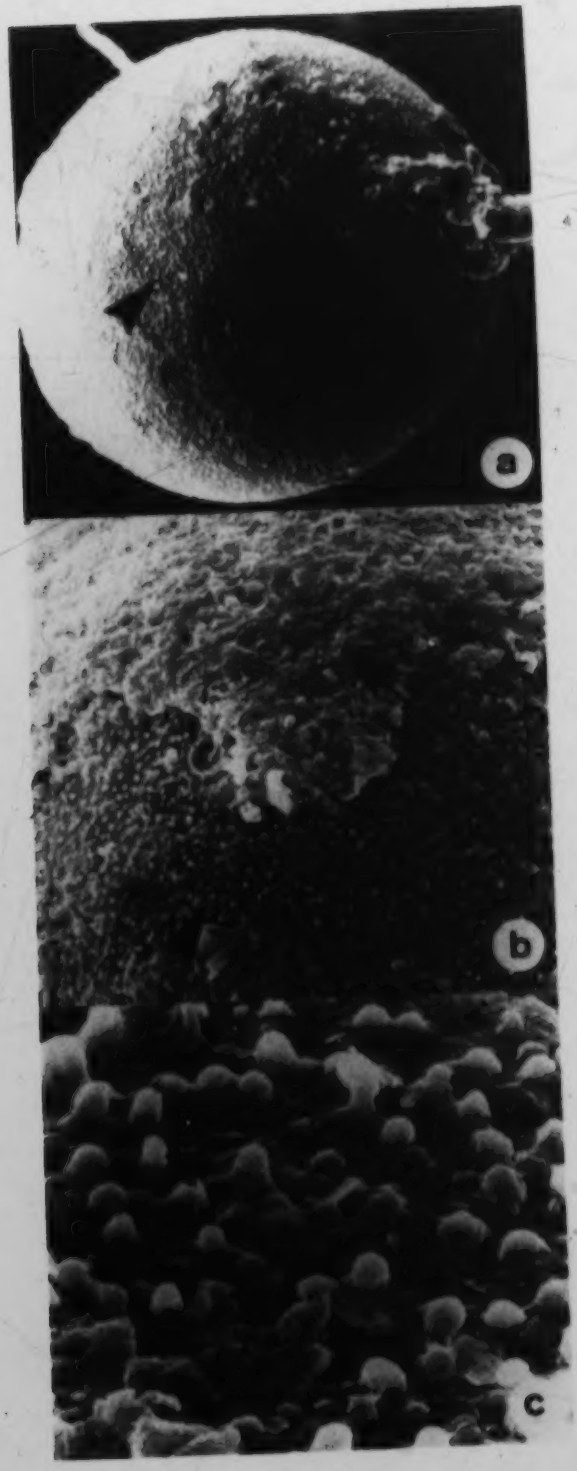
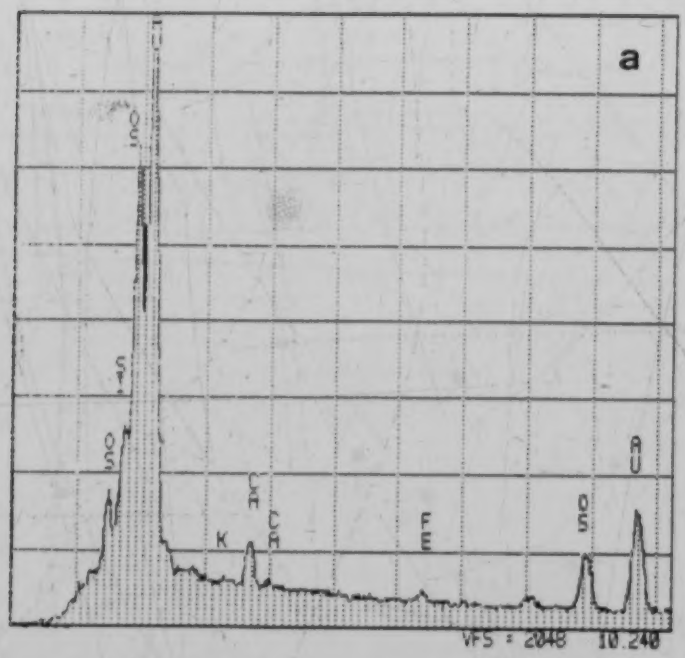

a
b
c

Figure II-10. X-ray spectra of spore walls of *Glomus pansihalos*.
Fig. II-10a. X-ray spectrum of outer expanding wall. Some calcium is detected in this wall.
Fig. II-10b. X-ray spectrum of laminate wall. Note the greater level of calcium in this wall as opposed to the outer wall.


a
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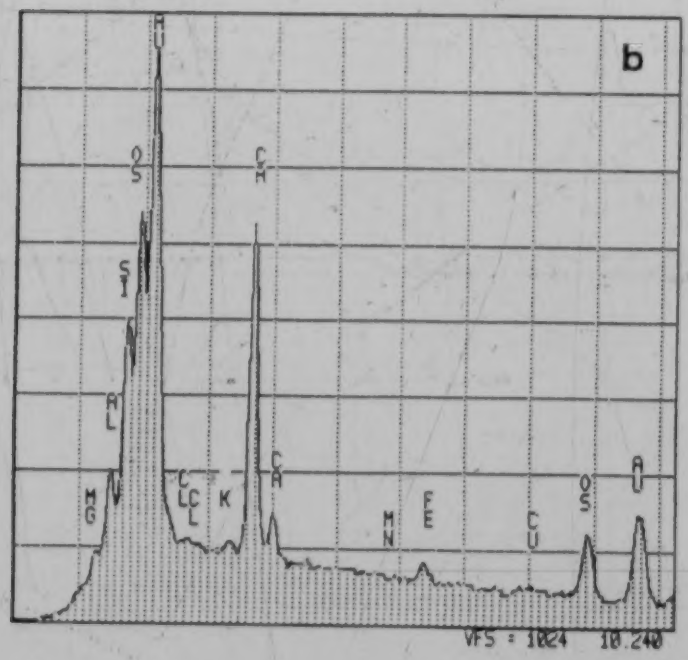
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VFS = 1024 10.240

Figure II-11. Light micrographs of *Glomus tenue* spores.
Fig. II-11a, 11b. Spores of *G. tenue* in association with barley roots.
Mature spores are 9-15 µm in diameter. II-11a X 1525, II-11b X 1525.

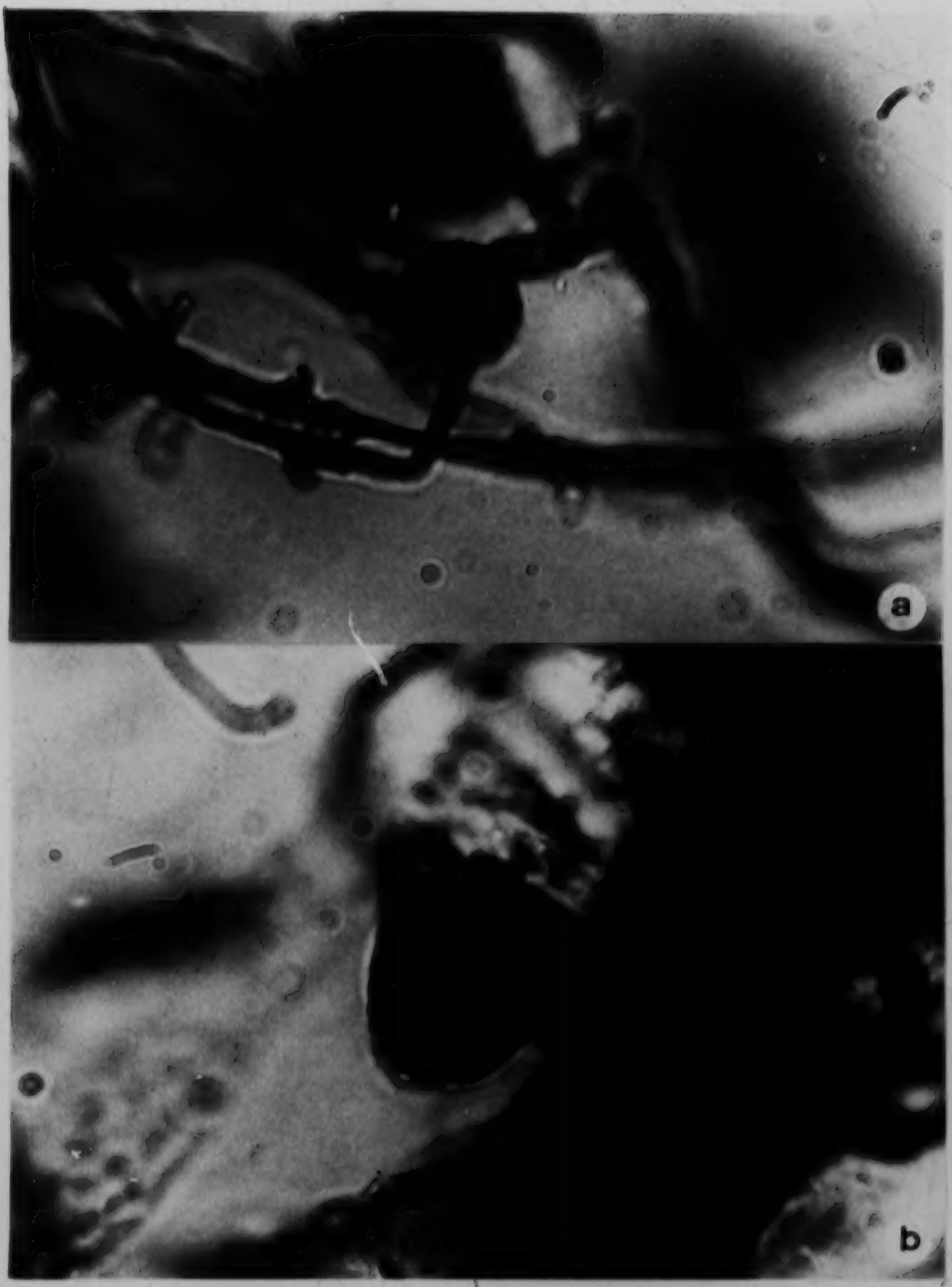

a
b

Figure II-12. Light micrographs of *Entrophospora infrequens* spores.
Fig. II-12a, 12b. Mature spores with the vesicle (sporiferous saccule) attached (arrow), and spore enclosed by the hyaline wall of the vesicular stalk (large arrowheads). The ornamented wall bears vacuolated spines (small arrowheads). II-12a X 190, II-12b X 525.

a
b

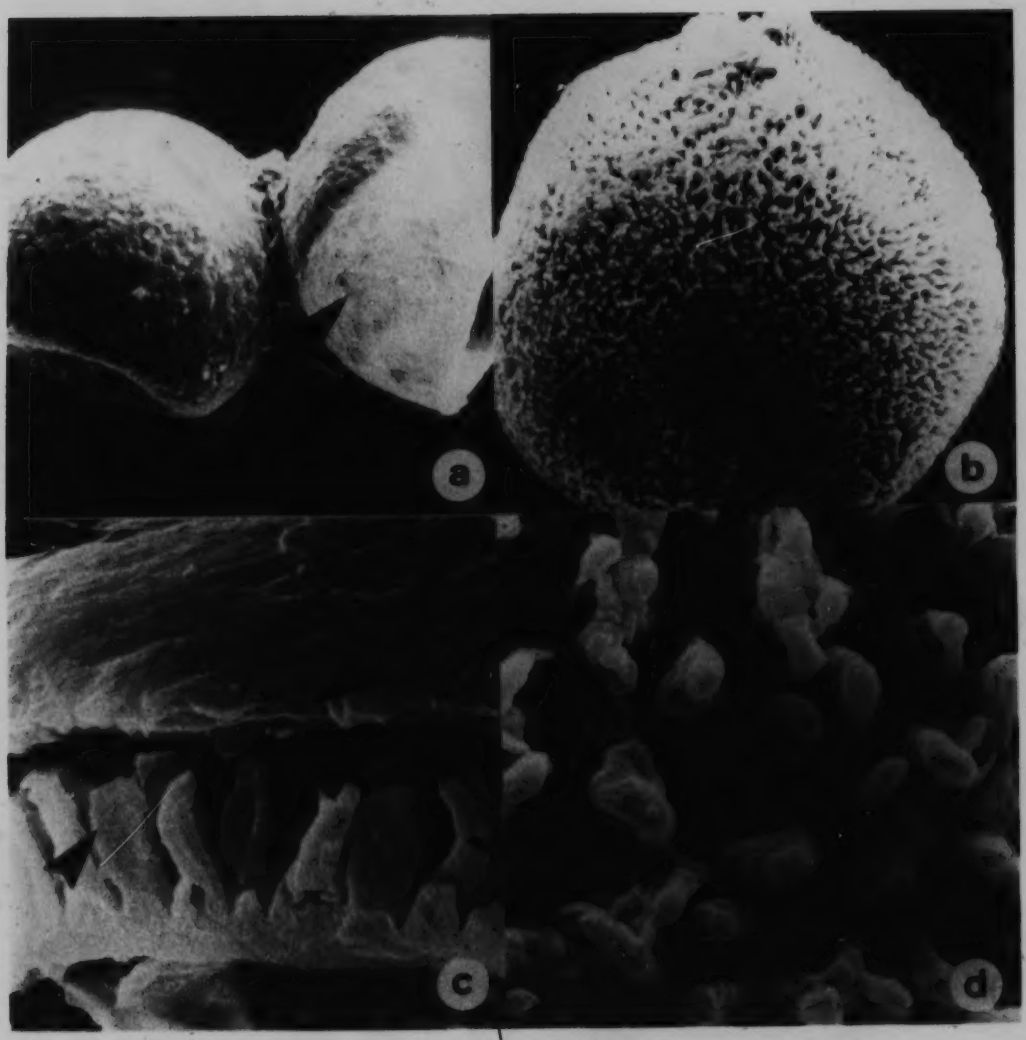
Figure II-13. SEM photographs of *Entrophospora infrequens* spore and spore walls.

Fig. II-13a. Typical spore with vesicle (sporiferous saccule) remaining attached. X 400.

Fig. II-13b. Mature spore, with wall from vesicular stalk absent. Note that the spore wall is highly ornamented, consisting of spines. X 900.

Fig. II-13c. Cross-sectional view of wall from vesicular stalk (arrow) surrounding the ornamented wall of the spore (arrowhead). X 6000.

Fig. II-13d. Enlarged view of spore surface covered with spines. X 8500.


a
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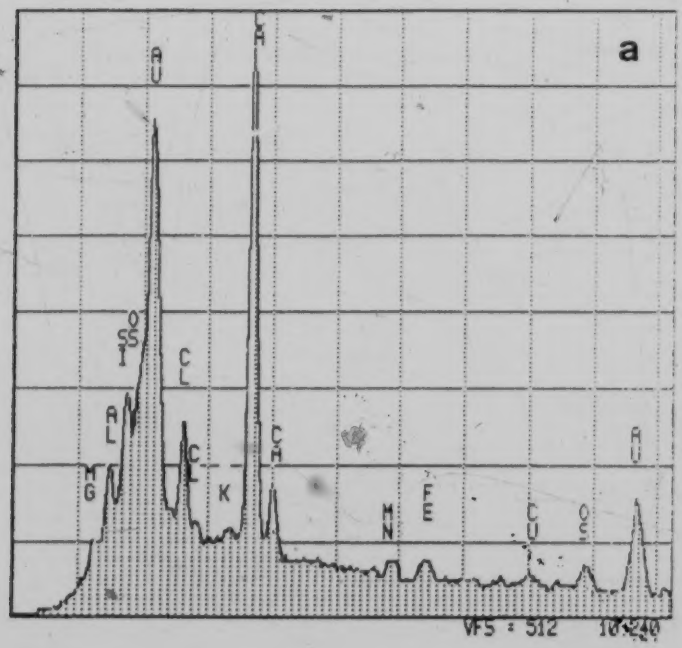
c
d

Figure II-14. X-ray spectra of spore wall of *Entrophospora infrequens*.
Fig. II-14a. X-ray spectrum of wall of vesicle. Calcium was detected in the wall. Note that the VFS = 512.
Fig. II-14b. X-ray spectrum of ornamented wall. This wall contains similar levels of calcium as the vesicular-wall. Note that the VFS = 1024. Therefore, when comparing the 2 spectra, the peak obtained for calcium in Fig. II-14a will approximately the same as that seen in Fig. II-14b.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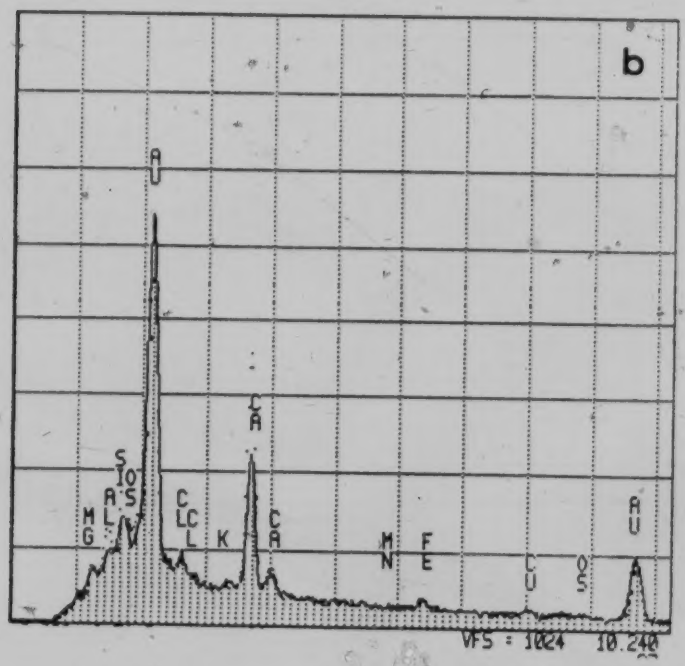
VFS = 512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VFS = 1024
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Figure II-15. Light micrographs of *Glomus mosseae* spores.
Fig. II-15a. Mature spore collected from Lacombe. X 490.
Fig. II-15b. Mature spore collected from Fairview. X 515.

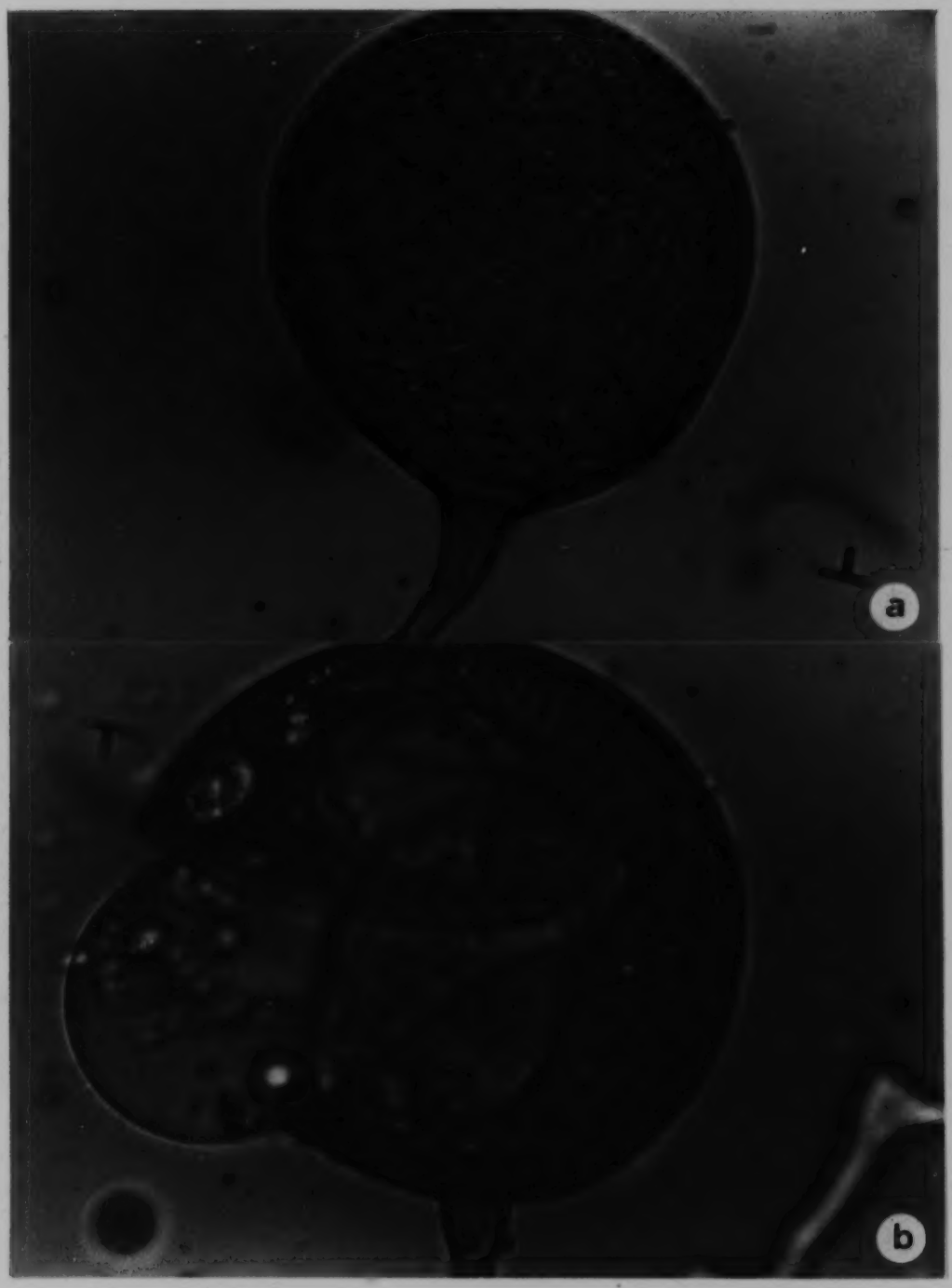
a
b

Figure II-16. Light micrographs of *Glomus mosseae* spore obtained from INVAM.

Fig. II-16a. Sporocarp containing spherical spores. X 600.

Fig. II-16b. Large numbers of spores outside alfalfa roots. X 195.

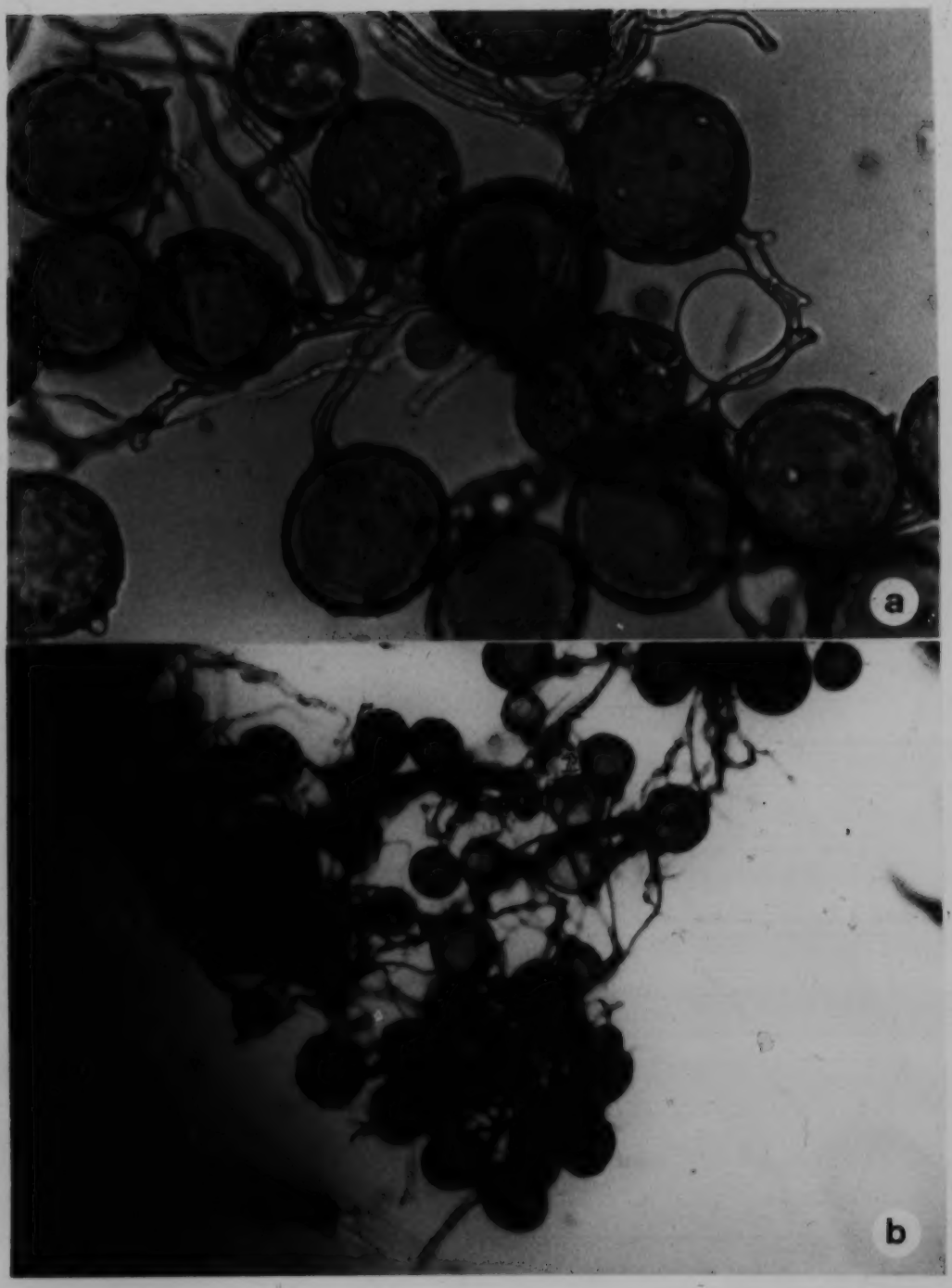
a
b

Figure II-17. Light micrographs of *Glomus intraradices* spores.

Fig. II-17a. Mature spore. Note the presence of a hyaline outer wall (arrowhead). X 880.

Fig. II-17b. Cleared root of alfalfa containing numerous spores. X 195.

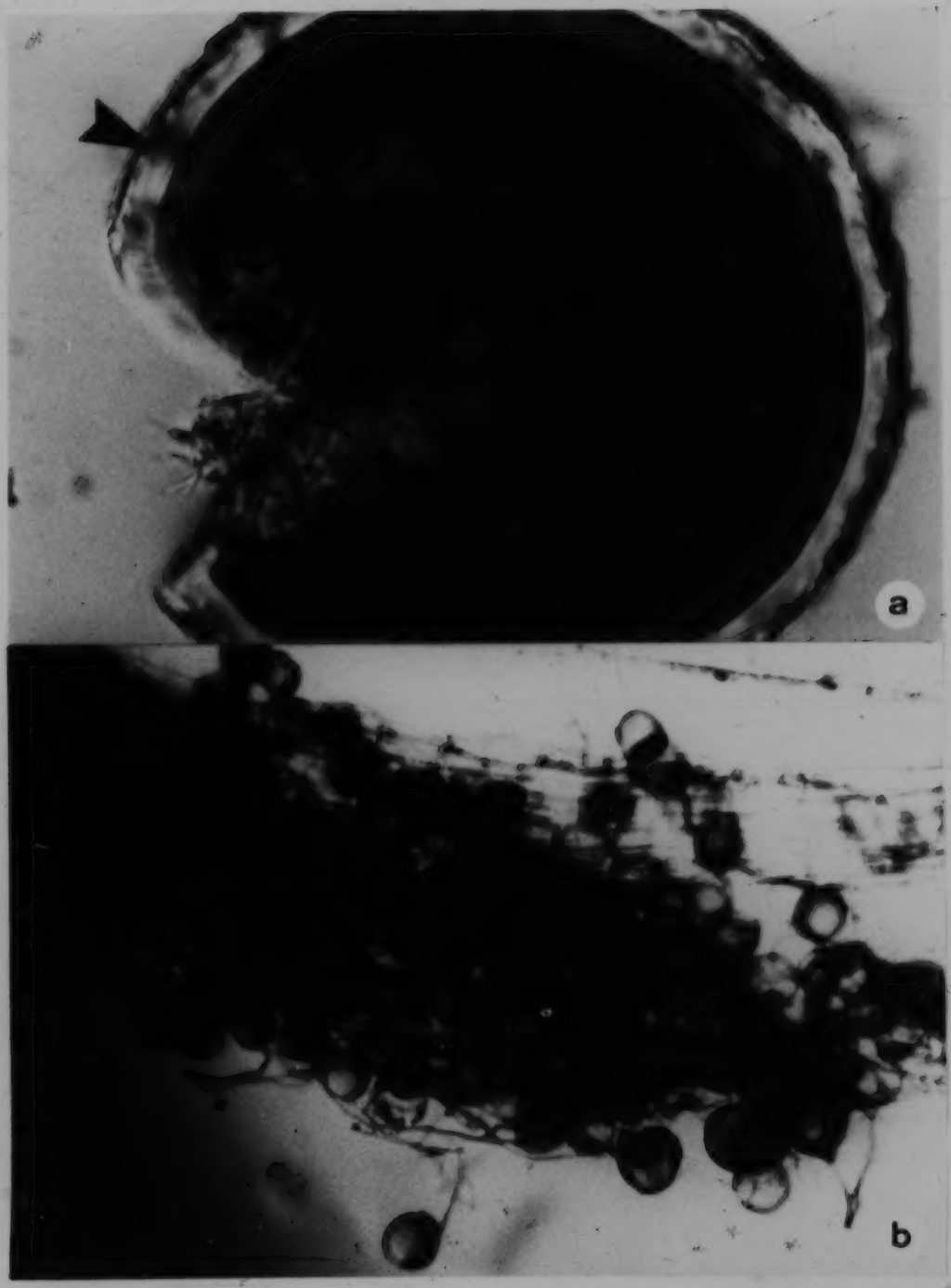
a
b

Figure II-18. SEM photographs of *Glomus mosseae* and *Glomus intraradices* spores.

Fig. II-18a. Spore of *G. mosseae* collected from field soil at Lacombe. Note the slightly flared sporogenous hypha (arrowhead). X 2200.

Fig. II-18b. Spore of *G. mosseae* obtained from INVAM. X 1600.

Fig. II-18c. Spore of *G. intraradices*. Note the agglutination of soil particles to the spore wall surface (arrowheads). X 850.

Fig. II-18d. View of the inside of a *G. intraradices* spore (arrow). X 1900.

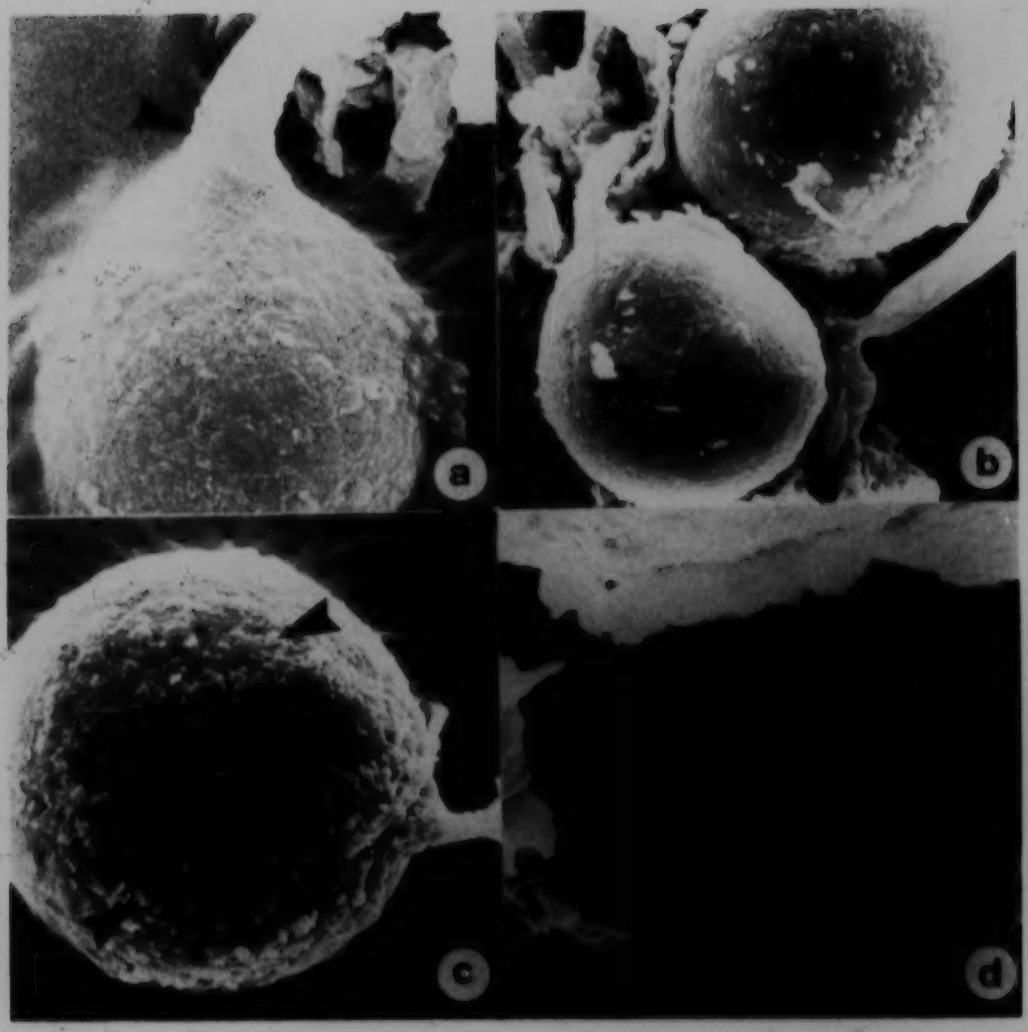
a
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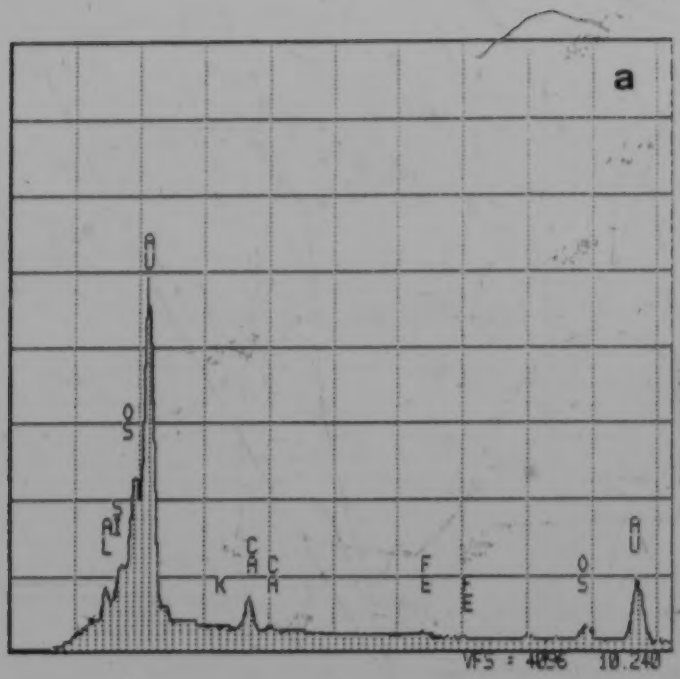
Figure II-19. X-ray spectra of spore wall of *Glomus mosseae*.

Fig. II-19a. X-ray spectrum of spore wall of *G. mosseae* isolated from soil at Lacombe. Note the presence of calcium in the wall.

Fig. II-19b. X-ray spectrum of spore wall of *Glomus mosseae* obtained from INVAM. Levels of calcium detected in the wall were similar to those detected in the walls of the Alberta isolate.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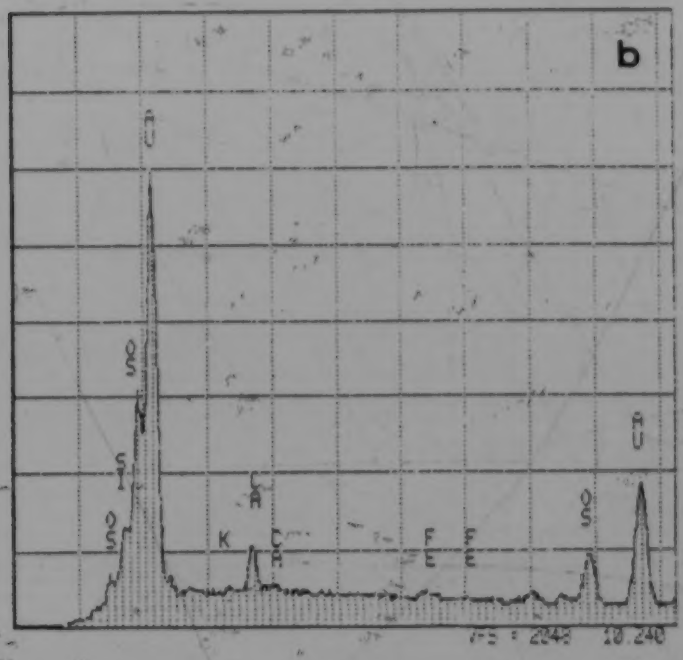
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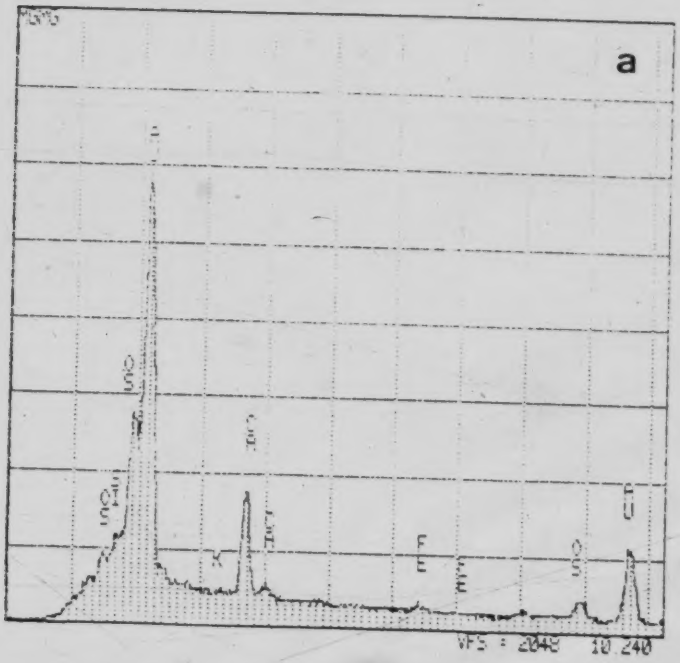
VFS = 2048 10.240

Figure II-20. X-ray spectra of spore walls of *Glomus intraradices*.
Fig. II-20a. X-ray spectrum of outer wall of *G. intraradices*. Note the presence of calcium in the wall.
Fig. II-20b. X-ray spectrum of innermost wall of *G. intraradices* (see Fig. II-18d). Calcium contained in this wall was relatively greater that than detected in the outer wall.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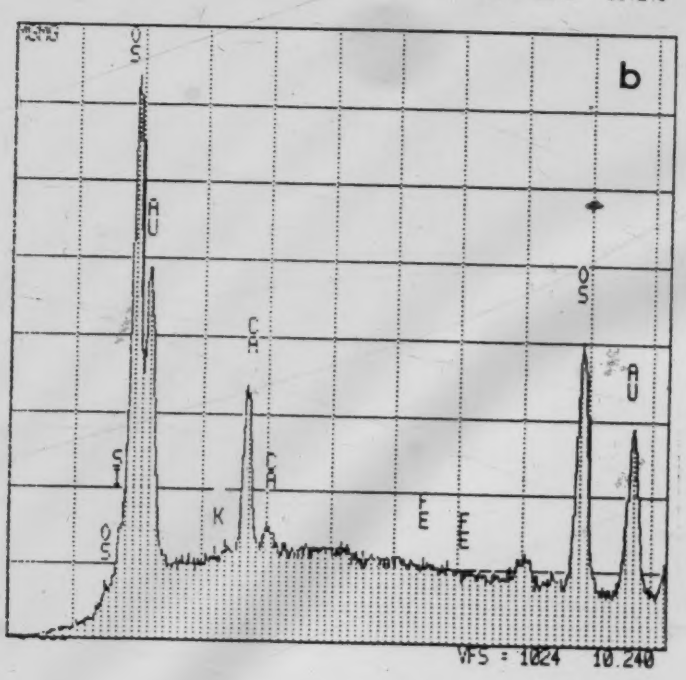
b
VFS = 1024 10.240

Figure II-21. X-ray spectrum of a soil particle agglutinating to the spore wall surface of *Glomus intraradices* (see Fig. II-18c). Note the predominance of silicon, in addition to aluminum, iron and calcium in the soil particle.

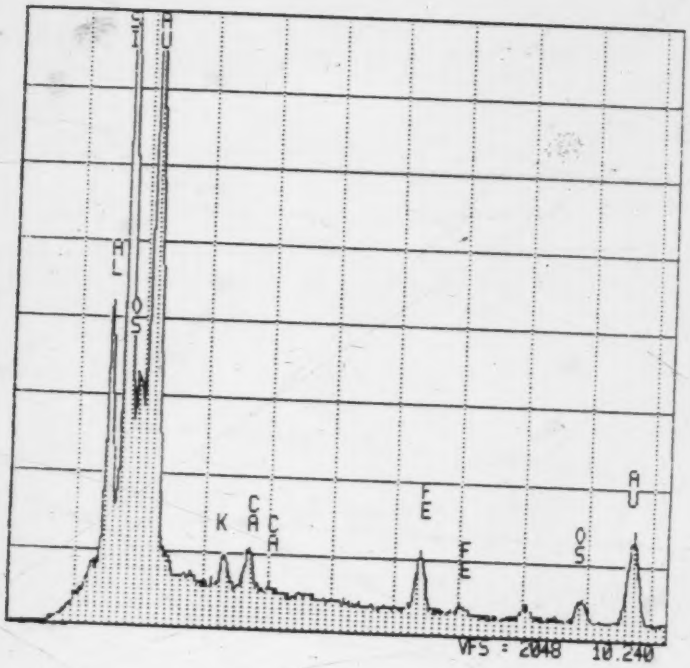
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Figure II-22. SEM photographs of *Bipolaris sorokiniana* spore and hyphae and barley root and root hairs.

Fig. II-22a, 22b. Cigar-shaped spore (II-22a) and hyphae (II-22b) of *B. sorokiniana*. II-22a X 3900, II-22b X 700.

Fig. II-22c, 22d. Barley root (II-22c) and root hairs (II-22d).

II-22c X 350, II-22d X 600.

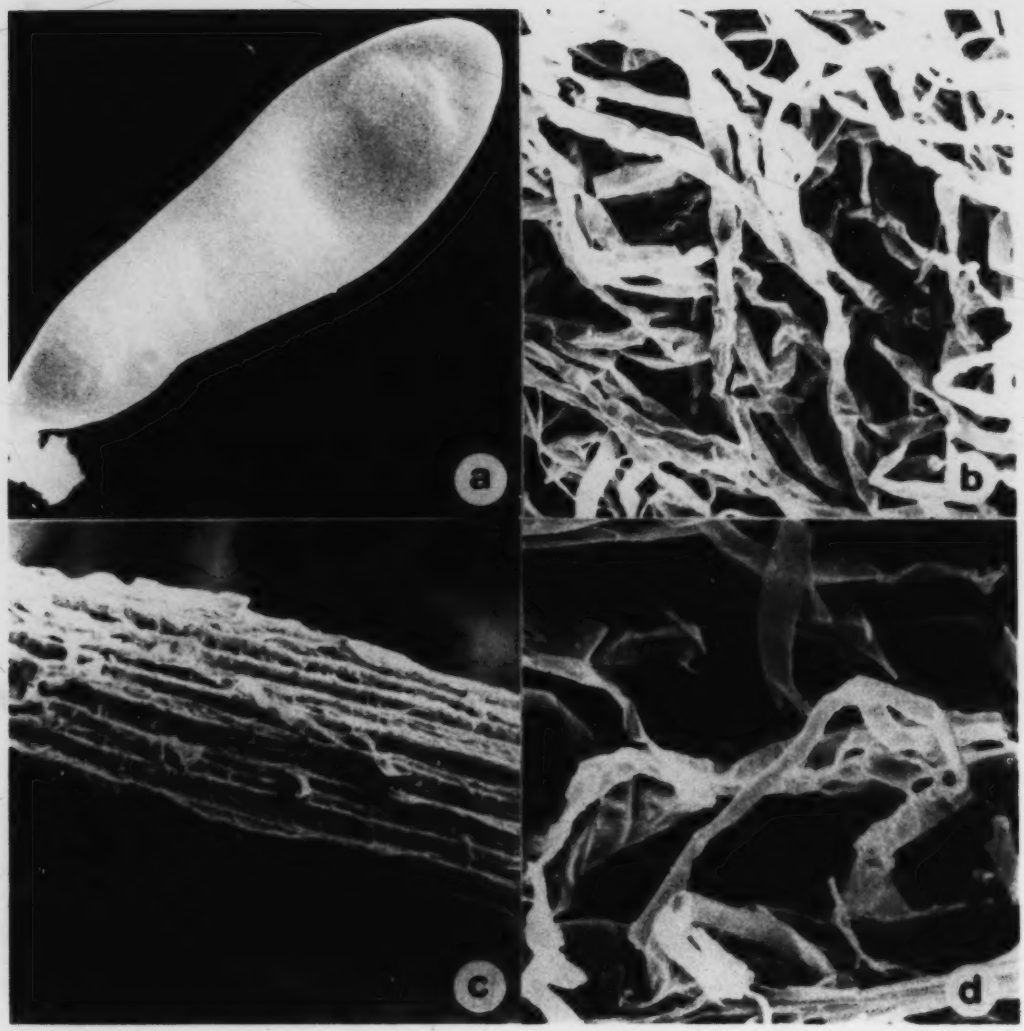
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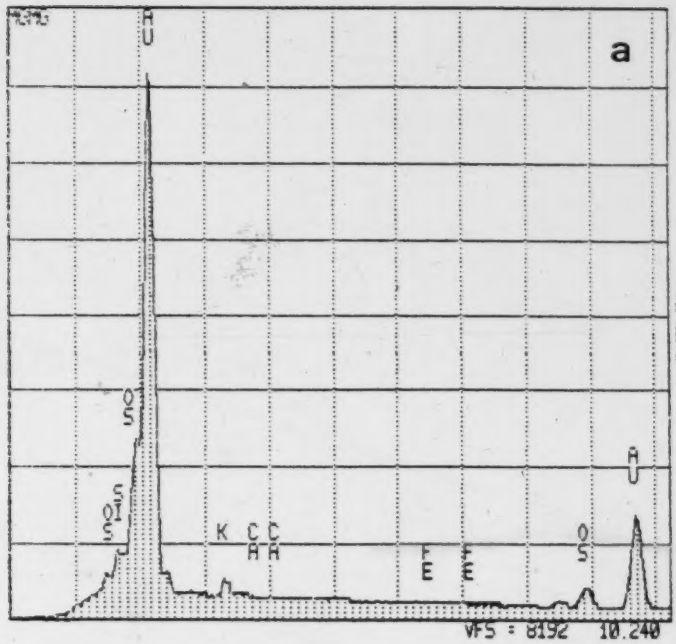
Figure II-23. X-ray spectra of *Bipolaris sorokiniana* spore wall and hyphal wall.

Fig. II-23a. X-ray spectrum of spore wall. Note that no calcium was detected. Potassium was detected in the wall.

Fig. II-23b. X-ray spectrum of hyphal wall. Note that no calcium was detected. Potassium was detected in the hyphal wall.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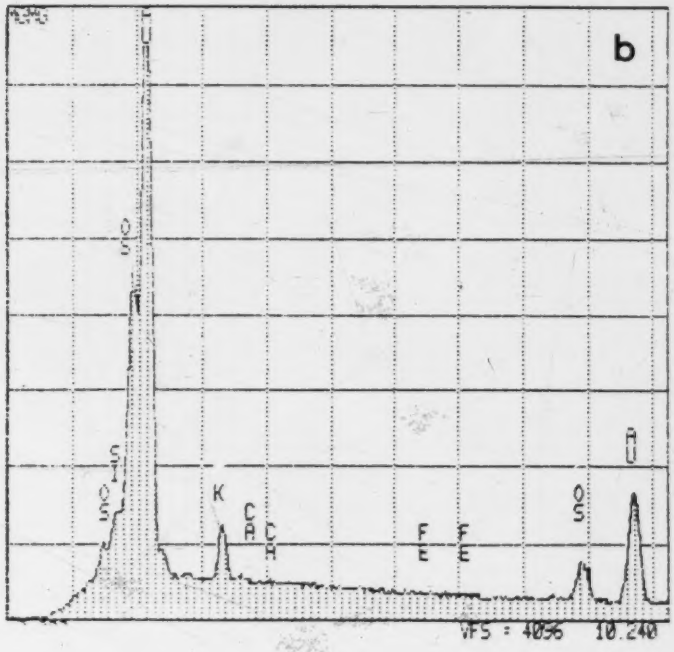
VFS = 8192
10.240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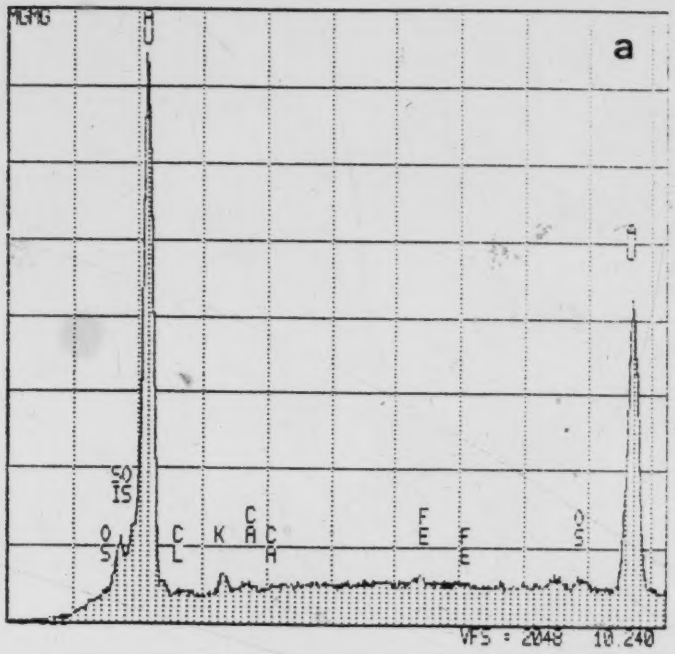
VFS = 4096
10.240

Figure II-24. X-ray spectra of barley root and root hair surfaces.
Fig. II-24a. X-ray spectrum of barley root surface. Calcium was detected in relatively low amounts. Potassium was also detected on the root surface.
Fig. II-24b. X-ray spectrum of suface of barley root hairs. Calcium and potassium were detected. Note that calcium occurred at relatively higher levels in root hairs than in roots.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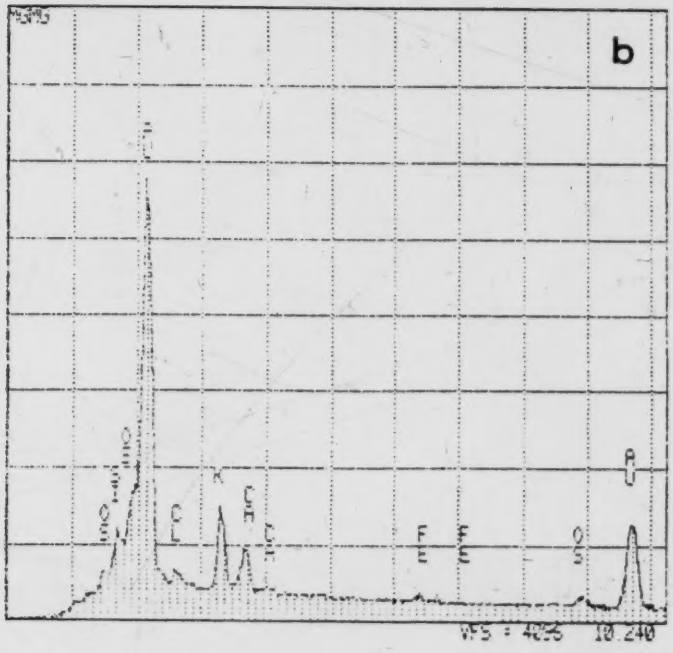
VFS = 2048 10.240


b
VFS = 4095 10.240

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Chapter III

Susceptibility of Various Barley Cultivars to VA Mycorrhizal Fungi[1]

A. Introduction

Vesicular-arbuscular (VA) mycorrhizal fungi are ubiquitous fungi with a worldwide distribution and have the ability to become associated with plants of economic importance such as the cereal crops (Khan, 1975; Owusu-Bennoah and Mosse, 1979; Jensen and Jakobsen, 1980; Powell *et al.*, 1980; Powell, 1981; Jensen, 1982). The ability of VA mycorrhizal fungi to colonize plant roots can vary among plant species (Boyetchko and Tewari, 1990). Since there exists genetic variability among cultivars to pathogens, variability may also exist in the susceptibility of plant cultivars to VA mycorrhizal fungi (Azcon and Ocampo, 1981; Krishna *et al.*, 1985; Heckman and Angle, 1987; Toth *et al.*, 1990). Azcon and Ocampo (1981) suggested that root exudation was a determining factor in susceptibility of wheat cultivars to VA mycorrhizal infection. In addition, Mercy *et al.* (1990) showed that the ability of cowpea plants to become infected by VA mycorrhizal fungi is a heritable trait and is host-dependent.

Enhanced growth and yield of barley by VA mycorrhizal fungi often results due to increases in uptake of nutrients such as phosphorus, nitrogen, zinc, and copper (Saif and Khan, 1977, Powell *et al.*, 1980; Powell, 1981; Jensen, 1982). However, information regarding the susceptibility of various barley cultivars to VA mycorrhizal infection is limited since previous studies have usually included one cultivar only. Therefore, field and greenhouse trials were undertaken to determine the relative susceptibility of commercially grown Canadian barley cultivars to VA mycorrhizal fungi. The barley cultivars studied were selected on the basis of their susceptibility to common root rot of barley caused by *Cochliobolus sativus* (Ito & Kurib.) ex Dastur.

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[1] A version of this chapter has been submitted for publication. Boyetchko, S.M., and J.P. Tewari. 1991. Susceptibility of various barley cultivars to VA mycorrhizal fungi. Plant and Soil (submitted)

B. Materials and Methods

Eight barley cultivars were chosen based on their susceptibility/resistance to *C. sativus* : Bonanza and Samson (moderate resistance), Klondike and Gateway 63 (intermediate resistance), and Olli, Johnston, Galt, and Klages (susceptible). The barley was grown at the University of Alberta Research Station in 1986, 1987, and 1988. The experimental design was a complete randomized block with four replicates; each cultivar was seeded with a four-row seed drill. The middle two rows were sampled in each treatment while the outside rows were left as guard rows. Root samples were collected when the barley reached Zadoks growth stage 50 (Totterman and Makepeace, 1979) and placed in formalin-acetic acid-alcohol (FAA) until such time that they could be examined for VA mycorrhizal root colonization.

In addition, barley cultivars grown at the Agriculture Canada Research Station at Lacombe , Alberta were assessed for VA mycorrhizal root colonization. Roots of twenty-two barley cultivars in 1986 and eighteen cultivars in 1987 were collected at Zadoks growth stage 94 (Totterman and Makepeace, 1979) and stored in FAA until they could be assessed for VA mycorrhizal root colonization.

Assessments for VA mycorrhizal fungi at both sites were based on the colonization of barley roots by indigenous VA mycorrhizal fungi.

Glomus dimorphicum Boyetchko & Tewari was originally isolated from a barley field in Alberta (Boyetchko and Tewari, 1986). Cultures were maintained in the greenhouses of the Department of Plant Science at the University of Alberta using alfalfa as a host plant. Plants were grown in steam sterilized sand:soil (3:1) which had an initial level of phosphorus of 6 ppm sodium bicarbonate extractable phosphorus. *Glomus intraradices* Schenck & Smith and *G. mosseae* (Nicol. & Gerd.) Gerd. & Trappe were obtained from the International Culture Collection of VA Mycorrhizal Fungi (INVAM) at the University of Florida, Gainesville from Dr. N.C. Schenck were also grown in a similar fashion. After 4 to 6 months, roots were removed from the soil and soil containing spores and hyphae of the respective VA mycorrhizal fungal species were used as inoculum immediately after harvesting.

The eight barley cultivars used in the field trials at the University of Alberta farm were also separately inoculated with soil containing approximately 300 spores of *Glomus dimorphicum* , *G. intraradices* , and *G. mosseae* per pot and grown in

the greenhouse. For each treatment, fifteen seeds of each cultivar were sown in 15 cm pots, and plants were subsequently thinned to ten plants per pot. A control in which no VA mycorrhizal fungal spores were added was used for comparison. The experiment consisted of four replicates. Soil mix consisted of sand:soil (3:1), and plants were fertilized weekly with 100 ml Hoagland's solution minus phosphorus, in addition to daily watering. The plants were grown in the greenhouse with a photoperiod of 16 hours and a maximum light intensity of 500 $\mu E/m^2/sec$. Day temperatures were approximately 25-27 C and night temperatures were maintained at 20 C. After eight weeks, the plants were harvested at Zadoks growth stage 50 (Totterman and Makepeace, 1979), shoot and root dry weights recorded, and root subsamples collected and stored in FAA until mycorrhizal assessment could be conducted. The experiment was repeated once and during this run, the plants were allowed to reach maturity, the seed was subsequently collected and yield and weight of 100 seeds (hundred kernel weight) were recorded. The shoot and root weights, yield and hundred kernel weights were based on 10 plant samples per treatment.

Roots collected from the field and greenhouse trials were washed with water after storage in FAA, cleared in 10% KOH, bleached in alkaline H_2O_2, acidified in 1% HCl, and stained in trypan blue lactic acid staining solution (Phillips and Hayman, 1970). The roots were then placed onto slides and VA mycorrhizal root colonization assessed using the grid-line intersect method (Giovannetti and Mosse, 1980) using a stereoscopic microscope. When more detailed observation was desired, the roots were examined under a compound light microscope.

Relationship between VA mycorrhizal root colonization and each plant growth parameter (i.e. shoot weight, root weight, yield and hundred kernel weight) was determined by regression analysis. Since growth parameters among cultivars are inherently different, the shoot and root weights, yield and hundred kernel weight were set at 100 for nonmycorrhizal control plants, and relative growth for VA mycorrhizal plants compared against this standard. Hence, regression analysis of each growth parameter with VA mycorrhizal root colonization were conducted using relative shoot and root weights, yield, and hundred kernel weight.

C. Results

Results from the field trials conducted at the University of Alberta farm revealed that indigenous VA mycorrhizal root colonization was low (Table III-1).

No significant differences in root colonization among the eight barley cultivars were observed in 1986 and 1988 due to large variation among the replicates. However, in 1987, significant differences were seen among the cultivars, with cultivar Olli showing the highest level of root colonization at 6.9% while cultivar Klages showed the lowest level of root colonization of 1.8% (Table III-1).

The levels of root colonization by indigenous VA mycorrhizal fungi at the Lacombe Research Station in 1986 and 1987 were very low (Table III-2). Also, no significant differences in VA mycorrhizal root colonization among all barley cultivars examined were observed (Table III-2). The highest level of root colonization was observed in the cultivar Heartland in both 1986 and 1987 at 7.2% and 6.1%, respectively (Table III-2). The lowest levels of VA mycorrhizal root colonization were observed in Argyle, in 1986, at 1.7% and Klondike, in 1987, at 2.0%.

Significant differences in VA mycorrhizal root colonization among barley cultivars to the three *Glomus* species were observed (Table III-3). Infection levels ranged from 1.8% in Galt inoculated with *G. mosseae* to 18.5% in Bonanza inoculated with *G. intraradices*. Differences in the ability of each VA mycorrhizal fungal species to colonize barley cultivars were also observed (Table III-3). Within each cultivar, significant differences in the level of root colonization by each *Glomus* species were seen (Table III-3). For example, root colonization of Bonanza roots by *G. dimorphicum* was 8.0%, which was significantly different than *G. intraradices* at 18.5% and *G. mosseae* at 4.1% (Table III-3). Other examples of significant differences in ability of the *Glomus* species to colonize a particular cultivar are seen in the cultivars Gateway 63 and Galt. However, in Samson, Klondike, Olli, Johnston, and Klages, all three *Glomus* species were capable of colonizing the roots of each cultivar to a similar extent (Table III-3).

Information on effects of the three VA mycorrhizal fungal species on growth (i.e. shoot and root weights) and yield (i.e. yield and hundred kernel weight) are provided in Tables III-4 to III-7. Since each cultivar is inherently different with respect to growth and yield, the effects of the VA mycorrhizal fungi on these growth parameters were compared with the nonmycorrhizal control plants within the same cultivar. Shoot and root weights differed among *Glomus* species depending on the cultivar. In some cases, shoot weights were similar among nonmycorrhizal and mycorrhizal plants (e.g. Samson, Gateway 63, Galt, and Klages) (Table III-4). In some cultivars, shoot weights of mycorrhizal plants were lower than nonmycorrhizal control plants (e.g. Samson, Klondike, Olli, Johnston)

depending on the *Glomus* species (Table III-4). In other cultivars, inoculation with a VA mycorrhizal fungus resulted in higher shoot weights than nonmycorrhizal control plants (Table III-4). Similar findings were observed for root weights (Table III-5). In four cultivars (e.g. Bonanza, Klondike, Johnston, and Klages), plants inoculated with *G. mosseae* had significantly higher root weights than nonmycorrhizal control plants.

Results from yield and hundred kernel weights revealed similar trends as seen for shoot and root weights (Tables III-6 and III-7). Nonmycorrhizal and mycorrhizal plants had similar yields in some cultivars while in other cultivars, differences between nonmycorrhizal and mycorrhizal plants were observed (Table III-6).

Regression analyses relating VA mycorrhizal root colonization with shoot weight, root weight, yield and hundred kernel weight revealed a lack of correlation. Correlation coefficients were extremely low for all comparisons made.

D. Discussion

The results from the field trials indicated that the barley cultivars had low levels of VA mycorrhizal root colonization, both at the University of Alberta farm and at the Lacombe Research Station. In addition, there were no significant differences among all the barley cultivars tested in susceptibility to the indigenous VA mycorrhizal fungi, although there were some differences among the eight barley cultivars in 1987. It is possible that the appropriate VA mycorrhizal fungal species compatible with the barley cultivars tested were not present in the field soil. Powell *et al.* (1980) observed that indigenous VA mycorrhizal fungi infected barley at low levels (i.e. 15%). Jensen (1982) also observed low levels of root colonization by *G. constrictum* Trappe (i.e. 6%).

VA mycorrhizal assessment of the barley roots in the greenhouse trial showed significant differences among the cultivars in their susceptibilities to each VA mycorrhizal species. Other researchers have also observed varied susceptibility among cultivars to VA mycorrhizal fungi (Azcon and Ocampo, 1981; Krishna *et al.*, 1985; Heckman and Angle, 1987; Mercy *et al.*, 1990). Heckman and Angle (1987) tested fifteen soybean cultivars and showed that VA mycorrhizal root colonization varied significantly among the cultivars. Similar results were reported in cowpea (Mercy *et al* ., 1990) and pearl millet (Krishna *et al.*, 1985). It was suggested that VA mycorrhizal root colonization is dependent on

the host genotype (Krishna *et al.*, 1985). Our results from the greenhouse trials concur with their findings. Estaun *et al.* (1987) reported that VA mycorrhizal root colonization did not vary among pea cultivars. However, the authors only tested three cultivars and differences perhaps may have been observed if a larger selection of pea cultivars were tested. Duc *et al.* (1989) found that genetic resistance to VA mycorrhizal fungi exists and that at least three genes are involved in determining establishment of mycorrhizal fungi in plant roots. They further indicated that resistance of plants to VA mycorrhizal root colonization is not specific to one strain of mycorrhizal fungus because resistance was observed equally to *G. intraradices* and *G. mosseae*.

Significant differences were observed in the ability of each VA mycorrhizal fungal species to colonize barley cultivars as well as in the susceptibility of each cultivar to individual *Glomus* species. Estaun *et al.* (1987) also showed distinct differences in VA mycorrhizal root colonization among three *Glomus* species. Jensen (1982) also observed that *G. constrictum* colonized barley roots at a level of only 6%, while *G. fasciculatum* (Thaxter sensu Gerd.) Gerd. & Trappe isolate no. 185 and no. 0-1 colonized barley roots at levels of 57% and 64%, respectively. These results support the suggestion that in the field trials, the appropriate VA mycorrhizal fungal species, compatible with barley, were not present in the soil. It has also been reported that introduced VA mycorrhizal fungal species improve plant growth, yield, and nutrient uptake significantly over indigenous species (Powell *et al.*, 1980; Powell, 1981). Owusu-Bennoah and Mosse (1979) further suggested that indigenous fungi are not strategically placed below the germinating seed as is the case for VA mycorrhizal fungi introduced into the field and this would reflect the differences in levels of root colonization between indigenous and introduced VA mycorrhizal fungi.

The three *Glomus* species clearly showed significant differences in their effects on shoot and root weights, yield and hundred kernel weight within each cultivar of barley. The growth responses differed among each *Glomus* sp., depending on the barley cultivar. Fairweather and Parberry (1982) similarly showed differences in ability of VA mycorrhizal fungal species to improve shoot and root dry weights of tomato. In the current study, regression analysis indicated that the growth responses were not necessarily related to the level of VA mycorrhizal root colonization. Similar results were observed in barley with other VA mycorrhizal fungal species (Owusu-Bennoah and Mosse, 1979; Jensen, 1982). Although, in the present greenhouse study, equal amounts of inoculum were

applied to the soil, there were clear differences in the effectiveness of the different *Glomus* species. Graham *et al.* (1982) observed that the amount of extraradical hyphae produced by various VA mycorrhizal fungi differed among species. It is possible that the extraradical hyphae are playing a more important role with respect to nutrient uptake and effect on plant growth than the intraradical phase, which was measured in our study.

Results of this study showed no relationship between susceptibility to barley common root rot and susceptibility to VA mycorrhizal root colonization. Although Bonanza is moderately resistant to *C. sativus* , causal agent of barley common root rot, this cultivar showed the highest level of colonization by *G. intraradices* over the other cultivars. Heckman and Angle (1987) also showed that resistance to Phytophthora root rot was not related to the ability of VA mycorrhizal fungi to colonize the soybean cultivars. A more recent report correlated general disease resistance to VA mycorrhizal fungal resistance in maize (Toth *et al.*, 1990). The authors compared the resistance of thirteen maize inbreds to ten pathogens for their susceptibility to VA mycorrhizal fungi. Our results have compared the barley cultivars with susceptibility to only one pathogen. Future research should consider comparison of VA mycorrhizal susceptibility with susceptibility of plants to a greater number of soil-borne pathogens.

The results from field and greenhouse trials conducted in this study suggest a certain degree of host-specificity and that the host-mycorrhizal fungus genotypes may influence the effectiveness of the symbiosis between the host root and the VA mycorrhizal species. Since a particular VA mycorrhizal fungal species which is highly effective at improving growth and yield of one plant species or cultivar does not perform as effectively on another species or cultivar, caution should be exercised when general recommendations are made. More research is needed to evaluate various species of VA mycorrhizal fungi, their inoculum potential, and effectiveness. Differences in the rate of infection and spread have been suggested (Owusu-Bennoah and Mosse, 1979) and this is likely to influence the response of plant species and cultivars to VA mycorrhizal inoculation.

E. Tables

Table III-1. VA Mycorrhizal Root Colonization (%) of Barley Cultivars at the University of Alberta Research Station (Edmonton).

Cultivar	Susceptibility to *C. sativus* *	% VA Mycorrhizal Colonization 1986	1987	1988
Bonanza	low	5.9 a	2.2 bc	8.9 a
Samson	low	3.1 a	4.2 b	3.9 a
Klondike	intermediate	3.8 a	2.4 bc	3.3 a
Gateway 63	intermediate	2.1 a	2.1 bc	6.7 a
Olli	high	1.9 a	6.9 a	4.2 a
Johnston	high	3.0 a	2.1 bc	4.1 a
Galt	high	3.4 a	2.4 bc	6.2 a
Klages	high	3.4 a	1.8 c	4.6 a

There was no significant difference among cultivars in 1986 and 1988 as determined by analysis of variance (P=0.05).

Numbers followed by the same letter are not significantly different from one another (LSD at P=0.05).

* Susceptibility is based on the extent of lesioning of the subcrown internode by the pathogen.

Table III-2. Indigenous VA Mycorrhizal Root Colonization (%) of Barley Cultivars at Lacombe Research Station.

Cultivar[a]	% VA Mycorrhizal Colonization[b]	
	1986	1987
Samson	2.4	2.8
Argyle	1.7	2.0
Bonanza	2.4	2.5
Heartland	7.2	6.1
Klondike	1.8	2.0
Klages	4.7	4.2
Harrington	3.4	3.8
Leduc	2.9	2.8
Norbert	6.8	-
Betzes	4.0	-
Elrose	3.9	-
Conquest	4.8	5.2
Gateway 63	3.9	-
Abee	4.5	4.6
Fairfield	3.9	-
Empress	4.3	-
Diamond	2.0	2.3
Olli	3.4	3.0
Johnston	6.6	5.9
Jackson	5.4	4.9
Galt	3.6	3.9
Otal	5.2	4.8
Deuce	-	3.9
Duke	-	3.1

a. "-" indicates the cultivars not grown in the field during the year.

b. There was no significant difference in mycorrhizal colonization among cultivars as determined by analysis of variance (P=0.05).

Table III-3. Colonization (%) of Barley Roots by 3 *Glomus* species - Greenhouse Inoculation Trial

Cultivar	Control	*Glomus dimorphicum*	*Glomus intraradices*	*Glomus mosseae*
Bonanza	0.0 a	8.0 fg	18.5 h	4.1 bcde
Samson	0.0 a	7.0 defg	6.9 def	6.2 def
Klondike	0.0 a	7.9 efg	6.5 def	7.1 defg
Gateway 63	0.0 a	4.1 bcde	10.8 g	6.7 def
Olli	0.0 a	7.8 efg	7.9 efg	5.7 cdef
Johnston	0.0 a	2.4 abc	5.2 bcdef	3.5 bcd
Galt	0.0 a	3.7 bcd	6.6 def	1.8 ab
Klages	0.0 a	3.7 bcd	6.3 def	4.4 bcdef

Numbers followed by the same letter are not significantly different from one another (Student Newman Keul's at P=0.05).

Table III-4. Effect of VA Mycorrhizal Inoculation on Shoot Weights (g dwt)of Barley
Cultivars in Greenhouse Trial

Cultivar	Control	*Glomus dimorphicum*	*Glomus intraradices*	*Glomus mosseae*
Bonanza	10.30 bc	12.28 a	11.13 ab	8.73 c
Samson	8.25 a	6.03 b	6.95 ab	5.70 b
Klondike	8.83 ab	9.13 a	8.70 a	6.25 b
Gateway 63	7.48 ab	7.08 b	9.28 a	6.65 b
Olli	9.70 a	7.60 b	9.73 a	5.65 b
Johnston	8.48 a	7.65 a	7.60 a	5.58 b
Galt	7.68 a	6.88 a	7.20 a	6.13 a
Klages	7.50 ab	8.25 a	7.95 ab	6.28 b

Within each cultivar, values followed by the same letter are not significantly different from one another (LSD at P=0.05).

Table III-5. Effect of VA Mycorrhizal Inoculation on Root Weights (g dwt) of Barley Cultivars in Greenhouse Trial

Cultivar	Control	*Glomus dimorphicum*	*Glomus intraradices*	*Glomus mosseae*
Bonanza	8.68 b	12.70 b	9.88 b	19.65 a
Samson	5.33 a	5.55 a	3.73 a	6.43 a
Klondike	7.15 c	13.55 ab	10.38 bc	15.70 a
Gateway 63	5.18 a	5.05 a	5.55 a	6.58 a
Olli	7.50 a	7.75 a	8.30 a	7.68 a
Johnston	7.50 b	6.90 b	7.35 b	16.80 a
Galt	12.33 a	10.15 a	8.73 a	13.20 a
Klages	9.58 b	12.30 ab	7.48 b	14.93 a

Within each cultivar, values followed by the same letter are not significantly different from one another (LSD at P=0.05).

Table III-6. Effect of VA Mycorrhizal Inoculation on Yield (g) of Barley Cultivars in Greenhouse Trial

Cultivar	Control	*Glomus dimorphicum*	*Glomus intraradices*	*Glomus mosseae*
Bonanza	5.25 bc	4.83 c	7.63 a	6.55 ab
Samson	4.03 ab	3.58 b	5.15 a	4.40 b
Klondike	6.70 a	3.93 b	6.88 a	5.98 a
Gateway 63	4.60 a	3.65 a	4.33 a	4.88 a
Olli	4.45 a	3.75 a	4.58 a	4.68 a
Johnston	3.63 b	3.73 b	5.43 a	4.38 ab
Galt	4.20 ab	3.23 b	5.35 a	4.10 ab
Klages	3.83 a	3.58 a	4.15 a	4.35 a

Within each cultivar, values followed by the same letter are not significantly different from one another (LSD at P=0.05).

Table III-7. Effect of VA Mycorrhizal Inoculation on Hundred Kernel Weight (g) of Barley Cultivars in Greenhouse Trial

Cultivar	Control	*Glomus dimorphicum*	*Glomus intraradices*	*Glomus mosseae*
Bonanza	2.73 a	2.88 a	3.03 a	2.80 a
Samson	2.75 b	2.85 b	3.18 a	2.88 ab
Klondike	3.53 a	3.18 b	3.73 a	3.63 a
Gateway 63	2.45 a	2.28 a	2.50 a	2.53 a
Olli	2.70 a	2.80 a	2.73 a	2.70 a
Johnston	2.70 b	3.13 a	3.00 ab	2.83 ab
Galt	2.93 b	2.83 b	3.35 a	3.00 b
Klages	3.23 b	3.25 b	3.53 ab	3.78 a

Within each cultivar, values followed by the same letter are not significantly different from one another (LSD at P=0.05).

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Chapter IV

Biological Control of Common Root Rot of Barley by Three VA Mycorrhizal Fungi[1]

A. Introduction

Vesicular-arbuscular (VA) mycorrhizal colonization in plant roots can greatly influence the development of diseases caused by soil-borne pathogens (Schenck and Kellam, 1978; Dehne, 1982; Smith, 1988; Caron, 1989). Severity of disease may be decreased (Davis and Menge, 1980; Graham and Menge, 1982; Caron *et al.*, 1986a, 1986b), increased (Davis *et al.*, 1979) or not affected (Zambolin and Schenck, 1983; Bååth and Hayman, 1984) by the presence of VA mycorrhizal fungi. In nutrient deficient soils, VA mycorrhizal fungi increase the uptake of nutrients such as phosphorus by exploring a greater volume of soil which may not be readily available to the plant (Hayman, 1983). Some researchers thus believe that the reduction in the severity of certain diseases may be an indirect result of improved phosphorus nutrition in the plant by VA mycorrhizal fungi (Davis and Menge, 1980; Graham and Menge, 1982).

Common root rot, caused by *Cochliobolus sativus* (Ito & Kurib.) Drechsl. ex Dastur (imperfect stage *Bipolaris sorokiniana* (Sacc.) Schoem.) is one of the most prevalent diseases of barley in the Canadian prairies (Ledingham *et al.*, 1973; Piening *et al.*, 1976). Annual yield losses in spring barley during 1970-72 averaged 10.3% (Piening *et al.*, 1976). Barley plants infected by this pathogen may have fewer heads, tillers, and reduced yield (Ledingham *et al.*, 1973; Piening *et al.*, 1976). Verma *et al.* (1975) reported that common root rot developed more rapidly in wheat grown in low phosphorus soils than in soils containing high levels of phosphorus. The application of phosphorus fertilizer to stubble fields resulted in a significant reduction in incidence of barley common root rot (Piening *et al.*, 1983). Since VA mycorrhizal fungi increase the uptake of phosphorus by plants (Hayman, 1983), they may play a role in the suppression of barley common root rot in phosphorus deficient soils.

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[1]A version of this chapter has been submitted for publication. Boyetchko, S.M., and J.P. Tewari. 1991. Biological control of common root rot of barley by three VA mycorrhizal fungi. Can. J. Plant Pathol. (submitted)

This study was initiated to determine if VA mycorrhizal fungi play a role in suppressing barley common root rot caused by *B. sorokiniana* when phosphorus in soils is limited. In addition, the study was conducted to determine the effect of the interaction between VA mycorrhizal fungi and *B. sorokiniana* on barley growth and yield. A preliminary report on part of this work has been published (Boyetchko and Tewari, 1988).

B. Materials and Methods

Glomus dimorphicum Boyetchko & Tewari was originally isolated from a barley field in Alberta (Boyetchko and Tewari, 1986). Cultures were maintained on alfalfa plants grown in steam sterilized sand:soil (3:1) in the greenhouses of the Department of Plant Science at the University of Alberta. Cultures of *G. intraradices* Schenck & Smith and *G. mosseae* (Nicol. & Gerd.) Gerdemann & Trappe were obtained from the International Culture Collection of VA Mycorrhizal Fungi (INVAM) at the University of Florida, Gainesville from Dr. N.C. Schenck. These species were also maintained on alfalfa plants under the same conditions as those for *G. dimorphicum* . After four months, the roots were removed from the soil. The inoculum used in the experiment consisted of soil containing hyphae and spores of VA mycorrhizal fungi.

Cultures of *B. sorokiniana* were grown on potato-dextrose agar (PDA) for ten days. The conidia were harvested by washing the fungus from the plates with sterile distilled water and passing the suspension through a double layer of cheese cloth to remove the mycelium. Soil mix consisting of sand:soil (3:1) was inoculated with the resulting conidial suspension to obtain four different inoculum densities (0, 1, 2, and 4 conidia per cm^3 soil). A preliminary study had been undertaken to determine the inoculum densities required to obtain moderate to severe levels of disease using the cultivar Olli and the isolate of *B. sorokiniana* which were used in the experiments.

Barley (*Hordeum vulgare* L. cv. Olli) was selected for this study because this cultivar is relatively susceptible to common root rot disease (Anonymous 1985). The seeds were surface sterilized in 3% sodium hypochlorite for ten minutes, rinsed twice with sterilized distilled water, and blotted dry on sterilized paper towels. The barley was seeded in a soil mixture of sand:soil (3:1), which had an initial phosphorus concentration of 6 ppm sodium bicarbonate extractable phosphorus, at a depth of 6 cm in 15 cm pots. The plants were sown to such a

depth in order to ensure that infection by the pathogen would take place and to obtain subcrown internodes long enough to visually assess the internodes for disease severity. Fifteen seeds were initially placed in each pot and after emergence, thinned to ten plants per pot. The seeds were covered with 500 cm^3 soil containing conidia of *B. sorokiniana* at one of the four inoculum densities previously described. In addition, inoculum of *G. dimorphicum*, *G. intraradices*, or *G. mosseae* was placed below the barley seed. Inoculum consisted of soil containing hyphae and approximately 300 spores of each species per pot. Equal numbers of spores of each species were used separately in each pot. A control in which no VA mycorrhizal fungi were added to soil was used for comparison.

Plants were grown in the greenhouse, watered daily and fertilized once a week with 100 ml Hoagland's solution, minus phosphorus, per pot. The day temperatures were approximately 25-27 C and night temperatures were kept at 20 C. The photoperiod was maintained at 16 hours, with a maximum light intensity of 500 $\mu E/m^2/sec$. The experiment was conducted twice, with eight replicates per experiment, and results from both experiments were combined.

After eight weeks, the barley plants were harvested at Zadoks growth stage 50 (Totterman and Makepeace, 1979). For each pot (ten plant sample) disease severity ratings were obtained by visual inspection of the subcrown internodes and severity of lesions on the internodes recorded as either clean, slight, moderate or severe (Ledingham *et al.*, 1973). Using numerical values of 0, 2, 5, and 10 for the categories, respectively, disease severity ratings (DR) for each treatment were determined (Tinline and Ledingham, 1979), where

$$DR\ (\%) = \frac{\sum (\text{no. plants in category} \times \text{numerical value}) \times 100}{\text{Total no. plants} \times \text{maximum class value}}$$

The severity of lesion development, which is related to the level of discoloration on the subcrown internodes, has been used as an indicator of disease intensity and thus resistance of cultivars to common root rot disease (Tinline and Ledingham, 1979; Duczek *et al.*, 1985). Shoot and root dry weights were recorded after drying the materials at 70 C over a 24 hour period. A subsample of root was obtained from each treatment for VA mycorrhizal assessment. Roots were cleared and stained according to Phillips and Hayman (1970) and per cent VA mycorrhizal root colonization assessed using the grid-line intersect method (Giovannetti and Mosse, 1980).

Phosphorus content in the shoots was determined by dry ashing a subsample of the dried shoots, digesting in hydrochloric acid and sulfuric acid, and

colorimetrically determining phosphorus using sodium molybdate (Fiske and Subbarrow, 1925).

Yield data were obtained by conducting the experiment a third time in the manner previously described, and harvesting the plants at Zadoks growth stage 94 (Totterman and Makepeace, 1979). Seed was collected and yield tabulated, based on ten plants per treatment. The weight of 100 seeds (hundred kernel weight) was also recorded.

C. Results

Severity of common root rot, determined from the disease ratings, was significantly reduced by all three VA mycorrhizal fungi, even when the inoculum density of *B. sorokiniana* was four conidia per cm^3 soil (Fig. IV-1). *Glomus intraradices* and *G. mosseae* were more effective at reducing common root rot disease than *G. dimorphicum* (Fig. IV-1). There was also a significant effect of the pathogen on disease severity as the inoculum density increased (Fig. IV-1). Common root rot disease is seed-borne, as well as soil-borne. Therefore, disease in the controls may be the result of seed-borne inoculum, indicating that surface-sterilization of the seed was not effective in eradicating the pathogen (Fig. IV-1).

Roots were colonized similarly by all three *Glomus* species (Table IV-1). In addition, VA mycorrhizal root colonization in the barley roots was not affected by the presence of the pathogen. Shoot weights of barley inoculated with *G. dimorphicum* were significantly lower than the nonmycorrhizal control plants in the presence and absence of the pathogen (Table IV-1). The shoot weights of plants inoculated with *G. intraradices* and *G. mosseae* were similar to those in the control treatment (Table IV-1). However, in the absence of the pathogen, plants inoculated with *G. intraradices* had significantly lower shoot weights than the nonmycorrhizal control plants (Table IV-1). Increasing the level of *B. sorokiniana* inoculum in the soil did not significantly reduce the shoot weights or root weights (Table IV-1). In the absence of the pathogen, root weights of nonmycorrhizal control plants were significantly greater than plants colonized by *G. intraradices* and *G. mosseae* (Table IV-1). Plants inoculated with *G. dimorphicum* had similar root weights as controls, in the absence of the pathogen (Table IV-1). When the pathogen was present, root weights of the mycorrhizal and nonmycorrhizal barley plants were not significantly different from one another (Table IV-1).

Phosphorus concentration in shoots of the VA mycorrhizal barley plants was not significantly different from the nonmycorrhizal barley plants (Table IV-1). In addition, the presence of *B. sorokiniana* did not affect the phosphorus content of plants (Table IV-1).

The yield of barley plants inoculated with *G. dimorphicum* was significantly lower than plants inoculated with *G. intraradices* and *G. mosseae* and also generally lower than the nonmycorrhizal control plants (Table IV-2). The nonmycorrhizal plants had significantly lower yields than the *G. mosseae* inoculated plants when the pathogen inoculum was absent from the soil (Table IV-2). However, in the presence of the pathogen, nonmycorrhizal control plants and plants colonized by *G. intraradices* and *G. mosseae* had similar yields. The hundred kernel weights were affected by the presence of the VA mycorrhizal fungi (Table IV-2). Generally, plants inoculated with *G. dimorphicum* had lower hundred kernel weights than plants inoculated with *G. intraradices* , and *G. mosseae* when conidia of *B. sorokiniana* were not present in the soil. For the most part, though, no differences in hundred kernel weight were observed between nonmycorrhizal and mycorrhizal plants. In addition, there was no significant effect of *B. sorokiniana* on the yield and hundred kernel weight at any level of inoculum. Tillering did not occur in the barley plants in any treatment.

D. Discussion

The results from this study indicated that all three *Glomus* species were effective in suppressing the severity of barley common root rot. Results from a study conducted in Syria also revealed that the incidence of common root rot was significantly lower when inoculated with a *Glomus* species in one of the barley cultivars tested (Grey *et al.*, 1989). However, the authors only measured the incidence of infection in the subcrown internodes and not the intensity of the disease. In our study, the severity of lesioning on each subcrown internode and, therefore, an indication of disease intensity, was recorded according to methods outlined by Ledingham *et al.* (1973). It has been reported that disease incidence due to infection by *C. sativus* at various inoculum levels remains about the same between resistant and susceptible cultivars but that intensity of common root rot disease increases more on susceptible cultivars than on cultivars which are resistant (Duczek *et al.*, 1985). This also indicates that there is resistance to expansion of the lesion and not to initial infection in the resistant cultivars. Grey *et al.* (1989) also

did not indicate the relative resistance or susceptibility of the two cultivars tested nor the *Glomus* species used in their study. It was observed that *G. fasciculatum* (Thaxter) Gerd. & Trappe and *G. constrictum* Trappe improved phosphorus uptake and thus conferred tolerance of citrus to *Phytophthora parasitica* Dast. while *G. mosseae* and *Gigaspora margarita* Becker & Hall were less efficient at improving phosphorus uptake and hence elicited a resistance mechanism to the pathogen (Davis and Menge, 1981). Therefore, all four VA mycorrhizal fungi were effective in reducing disease but there were two different mechanisms of disease reduction. Our results, along with those of Davis and Menge (1981) showed that different VA mycorrhizal fungal species may confer different effects on plant growth and different levels of disease resistance or tolerance.

Our results showed that the pathogen did not affect root colonization by all three *Glomus* species tested. Caron *et al.* (1986b) also observed no effect of *Fusarium* on the root colonization by *G. intraradices* . Rempel and Bernier (1990) reported a suppression of common root rot disease of wheat by *G. intraradices* but the presence of *C. sativus* decreased colonization of wheat roots by *G. intraradices*. This report appears to be a preliminary study and it is unknown whether the experiment was conducted under field or greenhouse conditions. Other researchers have observed an effect of the pathogen on VA mycorrhizal root colonization (Kaye *et al.*, 1984; Caron *et al.*, 1986a). No explanation, though, has been given thus far for such a phenomenon.

Lack of increase in shoot and root dry weights of barley plants inoculated with the three *Glomus* species tested indicated that these VA mycorrhizal fungi did not stimulate growth of barley grown under low phosphorus levels used in this work. Shoot dry weights of barley inoculated with *G. dimorphicum* were lower than those of the nonmycorrhizal plants. A similar observation was made with plants inoculated with *G. intraradices*, in the absence of *B. sorokiniana*. It is possible that *G. dimorphicum* itself was acting parasitically and acting as a sink for the plant photosynthate, particularly under stress conditions caused by the presence of *B. sorokiniana*. Hetrick *et al.* (1984) showed that mycorrhizal fungi in corn grown under drought stress may act parasitically and thus impart no benefit in growth or nutrition over nonmycorrhizal corn. A previous study revealed that growth responses in barley are partially determined by the host genotype where various barley cultivars may show differences in growth response due to VA mycorrhizal fungi (Boyetchko and Tewari, 1991). Grey *et al.* (1989) similarly showed that the barley cultivar WI2291 had no increase in plant dry weight when

inoculated with a VA mycorrhizal fungus, but with no additional phosphorus applied. However, the cultivar Harmal did show an increase in plant dry weight. Caron *et al.* (1986b) found no improvement in growth of tomatoes when inoculated with *G. intraradices*, in the presence of *Fusarium oxysporum* Schecht f.sp. *radicies-lycopersici* Jarvis & Shoemaker. They concluded that the VA mycorrhizal fungus did not stimulate growth or replacement of diseased roots. Also, it has previously been suggested that a minimum level of phosphorus is required for a beneficial growth response to occur (Hayman, 1983; Grey *et al.*, 1989).

The yield and hundred kernel weight of the barley plants were not significantly reduced as the inoculum level of *B. sorokiniana* was increased. Although the cultivar Olli is considered susceptible to common root rot caused by *B. sorokiniana*, the rate of lesion development is not known. Verma (1982) reported that common root rot infection in a susceptible cultivar, which increases steadily throughout an entire season, will incur greater yield loss than that in a resistant cultivar which exhibits rapid development of disease near maturity. Our results showed no significant decrease in yield or hundred kernel weight with increasing disease severity. Furthermore, no differences in the number of heads or amount of tillering between treatments were observed. This was probably due to low soil phosphorus and/or the sandy texture of the soil mixture used in the experiments. The predominance of sand in the soil may have contributed to the lack of available nutrients (eg. phosphorus). It is not clear, though, if barley can grow in this type of soil under normal field conditions.

All plants were adequately supplied with water throughout the experiment. Duczek (1986) revealed that common root rot is more severe when plants are flooded, particularly at the 4-leaf stage, than unflooded. It is possible that in the present study, the barley plants were supplied with moisture levels adequate to contribute to common root rot severity at the early stage of plant development. In contrast, Ledingham *et al.* (1973) reported that higher disease ratings, and therefore, yield losses, were due to drought conditions. They further suggested that plants may tolerate high levels of disease without experiencing serious yield losses if soil moisture is adequate during the period of plant maturation.

Previous workers found that yield loss was not related to lower thousand kernel weight but to the reduction in the number of heads (Piening *et al.*, 1976; Duczek, 1984). Duczek (1989) observed a pronounced reduction in the number of tillers in wheat due to infection by *C. sativus* in the field. He further reported that

tiller production was limited and yield was reduced when infection by the pathogen occurred early in the season. Our results would support these observations because the barley plants were inoculated with the pathogen at the time of seeding which would thus allow for infection to take place very early in the development of the barley plants.

Although, the present study revealed a significant suppression of common root rot disease in barley by three species of VA mycorrhizal fungi, further studies should be conducted to explore the actual role of phosphorus in the interaction. Consideration should also be given to the timing and development of disease and at which point the disease development is suppressed by VA mycorrhizal fungi (Caron *et al.*, 1986b). An arbritrarily selected time of observation for the interaction may result in misleading conclusions. A selected sampling during the interaction between the pathogen and the VA mycorrhizal fungi over time may further reveal the rate of pathogen infection in the susceptible cultivar Olli. These studies could also elucidate whether suppression of disease is stable over time.

E. Tables, Figures, and Legends

Figure IV-1. Effect of increasing inoculum density of *B. sorokiniana* and inoculation of plants with *G. dimorphicum* (G. dim), *G. intraradices* (G. intr), or *G. mosseae* (G. mos) on disease (%). Controls consisted of plants without VA mycorrhizal fungi. Bar on the graph represents LSD at P = 0.05.

Figure IV-1

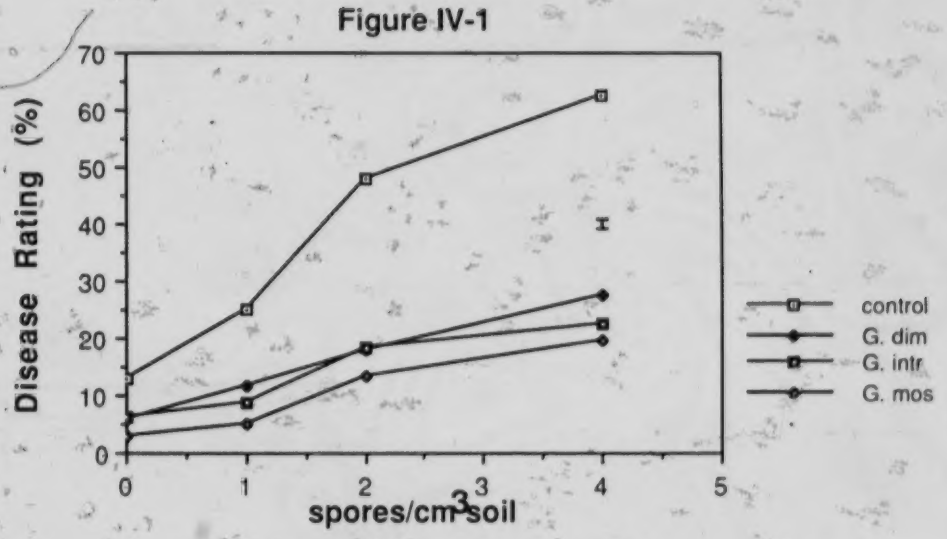
Disease Rating (%)
70
60
50
40
30
20
10
0
0
1
2
3
4
5
spores/cm3 soil
control
G. dim
G. intr
G. mos

Table IV-1. Effect of VA mycorrhizal fungi and *B. sorokiniana* on growth and yield of barley.

VA Mycorrhizal Fungus	*B. sorokiniana* conidia/cm^3 soil	Mycorrhizal Infection (%)	Shoot Wt (g)	Root Wt (g)	Phosphorus Conc. (mg/g)
control	0	0.0 b	6.9 ab	9.2 a	0.89 a
	1	0.0 b	7.0 a	7.2 bc	0.80 a
	2	0.0 b	6.3 bc	7.9 ab	0.88 a
	4	0.0 b	6.9 ab	7.3 abc	0.85 a
G. dimorphicum	0	7.4 a	5.3 d	7.4 abc	0.83 a
	1	9.3 a	5.6 d	6.6 bc	0.84 a
	2	7.3 a	5.7 cd	7.4 abc	0.76 a
	4	8.1 a	5.5 d	6.6 bc	0.87 a
G. intraradices	0	9.0 a	5.8 cd	7.1 bc	0.89 a
	1	7.7 a	6.7 ab	7.0 bc	0.87 a
	2	8.5 a	6.3 bc	7.0 bc	0.91 a
	4	9.2 a	6.5 ab	7.6 abc	0.85 a
G. mosseae	0	9.9 a	6.5 ab	6.5 bc	0.97 a
	1	9.8 a	6.7 ab	7.3 abc	0.91 a
	2	8.4 a	6.3 bc	6.4 bc	0.92 a
	4	9.6 a	6.5 ab	5.9 c	0.79 a
LSD at P=0.05		2.65	0.66	1.92	0.22

Table IV-2. Effect of VA Mycorrhizal Fungi and *B. sorokiniana* on Yield and Hundred Kernel Weight of Barley.

VA Mycorrhizal Fungus	*B. sorokiniana* conidia/cm^3 soil	Seed Yield (g)	Hundred Kernel Wt (g)
control	0	5.70 bc	2.80 bcd
	1	5.62 bcd	2.73 bcd
	2	5.28 cdef	2.85 abcd
	4	5.42 cde	2.69 d
G. dimorphicum	0	4.27 g	2.70 cd
	1	4.87 efg	2.81 bcd
	2	4.61 fg	2.87 abcd
	4	4.96 defg	2.79 bcd
G. intraradices	0	6.10 ab	2.94 ab
	1	5.55 bcde	2.91 abcd
	2	5.86 abc	2.86 abcd
	4	5.76 bc	2.92 abc
G. mosseae	0	6.55 a	2.95 ab
	1	5.54 bcde	3.06 a
	2	5.58 bcde	2.81 bcd
	4	5.89 abc	2.84 abcd
LSD at P=0.05		0.71	0.22

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Chapter V

Effect of Phosphorus on the Interaction Between VA Mycorrhizal Fungi and *Bipolaris sorokiniana* [1]

A. Introduction

Bipolaris sorokiniana (Sacc.) Shoem. causes common root rot of barley, one of the most important diseases of barley in the Canadian prairies (Ledingham *et al.*, 1973; Piening *et al.*, 1976). Annual yield losses in barley due to this disease can average 10.3% (Piening *et al.*, 1976). Low available soil phosphorus has been implicated as one of the factors contributing to the development of common root rot (Verma *et al.*, 1975; Piening *et al.*, 1983).

Several reports have shown significant reduction in disease severity attributed to inoculation of plants with vesicular-arbuscular (VA) mycorrhizal fungi (Davis and Menge, 1980; Graham and Menge, 1982; Caron *et al.*, 1986a, 1986b). Inoculation of barley with VA mycorrhizal fungi has also resulted in a significant suppression of common root rot disease (Grey *et al.*, 1989; Rempel and Bernier, 1990; Chapter IV). Some reports have shown that the suppression of various soil-borne pathogens by VA mycorrhizal fungi is regulated by improved phosphorus nutrition (Davis and Menge, 1980; Graham and Menge, 1982; Smith, 1988). The application of fertilizers, particularly phosphorus, inhibit the establishment of VA mycorrhizal fungi in plant roots (Hayman, 1983). However, it is possible that moderate levels of soil phosphorus may have no inhibitory effect on some of these fungi (Davis and Menge, 1980). Other reports have demonstrated that increased uptake of phosphorus is not the only mechanism by which VA mycorrhizal fungi reduce disease severity (Krishna and Bagyaraj, 1983; Kaye *et al.*, 1984; Caron *et al.*, 1986a).

The present study was carried out to determine if phosphorus has an effect on the interaction between VA mycorrhizal fungi and *B. sorokiniana*.

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[1] A version of this chapter has been submitted for publication. Boyetchko, S.M., and J.P. Tewari. 1991. Effect of phosphorus on the interaction between VA mycorrhizal fungi and *Bipolaris sorokiniana*. Phytopathology (submitted)

B. Materials and Methods

Cultures of *B. sorokiniana* were grown on potato-dextrose agar (PDA) at room temperature (about 22 C). The conidia were harvested after ten days by washing the agar plates with sterile distilled water and filtering the spore suspension through a double layer of cheese cloth to eliminate the mycelium. A soil mixture of sand:soil (3:1), which had an initial phosphorus concentration of 6 ppm sodium bicarbonate extractable phosphorus, was inoculated with the conidial suspension to obtain an inoculum density of 4 conidia per cm^3 soil. A preliminary study had revealed that this inoculum density would result in moderate to severe levels of common root rot disease using the cultivar Olli.

Cultures of the VA mycorrhizal fungi, *Glomus dimorphicum* Boyetchko & Tewari, *G. intraradices* Schenck & Smith, and *G. mosseae* (Nicol. & Gerd.) Gerdemann & Trappe, were maintained on alfalfa in steam sterilized sand:soil (3:1). *Glomus dimorphicum* was initially isolated from a barley field in Alberta (Boyetchko and Tewari, 1986). *Glomus intraradices* and *G. mosseae* were obtained from the International Culture Collection of VA Mycorrhizal Fungi (INVAM) at the University of Florida, Gainesville from Dr. N.C. Schenck. After four months, the alfalfa plants were harvested, the roots removed from the soil and the VA mycorrhizal inoculum added as soil containing hyphae and spores. Soil containing approximately 300 spores of each species were added to 15 cm pots, separately, as a layer on the soil. Another layer of soil mixture was placed on top of the inoculum before seeding. A control consisting of soil only with no VA mycorrhizal fungi added was used for comparison.

Barley (*Hordeum vulgare* L. cv. Olli) seed was surface sterilized in 3% sodium hypochlorite for ten minutes and rinsed twice with sterilized distilled water. The seed was placed on top of the VA mycorrhizal inoculum. Fifteen seeds were placed in each pot and plants were subsequently thinned to ten plants per pot. The seeds were covered with 500 cm^3 soil containing either 0 or 4 conidia of *B. sorokiniana* per cm^3 soil. This resulted in a seeding depth of 6 cm which was required to obtain subcrown internodes long enough for assessment of disease severity ratings. Four concentrations of phosphorus in the form of monocalcium phosphate, $Ca(H_2PO_4)_2 \cdot H_2O$, (0, 10, 20, and 50 µg phosphorus/g soil) were supplied to each pot once a week with the Hoagland's solution. The pots were also watered daily as needed. The experiment was conducted in the greenhouse with

day temperatures approximately 25-27 C and night temperatures maintained at 20 C. The photoperiod was 16 hours with a maximum light intensity of 500 $\mu E/m^2/sec$. The experimental design was a split plot consisting of 4 replicates.

After eight weeks, the plants were harvested at Zadoks growth stage 50 (Totterman and Makepeace, 1979). Common root rot severity ratings were conducted on roots by visually examining the subcrown internodes of each ten plant sample (Ledingham *et al.*, 1973; Tinline and Ledingham, 1979). The internodes were evaluated for the amount of discoloration by the pathogen and recorded as either clean, slight, moderate, or severe with numerical values of 0, 2, 5, and 10 assigned to each category, respectively, to obtain disease severity ratings (Tinline and Ledingham, 1979; Chapter IV). The amount of discoloration on the subcrown internode has been used as an indication of disease intensity (Tinline and Ledingham, 1979; Duczek *et al.*, 1985). Roots were cleared and stained (Phillips and Hayman, 1970) and per cent VA mycorrhizal colonization assessments were done using the grid-line intersect method (Giovannetti and Mosse, 1980).

Shoot dry weights, based on ten plant samples, were recorded after placing the plant material in an oven at 70 C for 24 hours. The phosphorus concentration in the shoots and roots was obtained by dry ashing plant material, digesting it in hydrochloric acid and sulfuric acid, and determining the phosphorus concentration colorimetrically (absorbance 650 nm) using the sodium molybdate method (Fiske and Subbarow, 1925).

C. Results

The presence of *G. dimorphicum*, *G. intraradices* , and *G. mosseae* in the soil resulted in suppression of common root rot, even at high levels of phosphorus (Fig. V-1). Both *G. intraradices* and *G. mosseae* were more effective in reducing common root rot than *G. dimorphicum* . The application of phosphorus was effective in decreasing root rot severity in the nonmycorrhizal plants, with all levels of phosphorus (i.e. 10, 20, and 50 µg phosphorus/g soil) having a similar disease rating (Fig. V-1). Phosphorus alone was not as effective in suppressing common root rot severity as the combination of VA mycorrhizal fungi and phosphorus however. Plants inoculated with *G. dimorphicum* had significantly lower disease severity ratings than nonmycorrhizal control plants in the presence of the pathogen, at all levels of phosphorus applied (Fig. V-1). Barley plants inoculated with *G. intraradices* and *G. mosseae* , in addition to *B. sorokiniana*, had significantly

lower disease severity ratings than nonmycorrhizal control plants and plants inoculated with *G. dimorphicum*. They also had similar disease severity ratings as nonmycorrhizal and mycorrhizal plants not inoculated with the pathogen (Fig. V-1). Some disease was present in both mycorrhizal and nonmycorrhizal plants, in the absence of the pathogen in the soil, at all levels of soil phosphorus. Barley common root rot disease is seed-borne as well as soil-borne. The presence of the pathogen on the seed indicated that seed-surface sterilization was not completely effective at eliminating the pathogen.

Shoot dry weights of barley significantly increased as the concentration of phosphorus applied to the plants increased (Fig. V-2). Shoot dry weights reached a maximum when 10 to 20 µg phosphorus/g soil were applied. In general, inoculation of barley with all three *Glomus* species, with the addition of phosphorus, resulted in higher shoot dry weights than nonmycorrhizal plants, in the presence and absence of *B. sorokiniana*, although not all treatments were significantly different. In the absence of the pathogen, shoot dry weights of plants inoculated with *G. intraradices* and *G. mosseae* were significantly higher than nonmycorrhizal control plants when 10 µg phosphorus/g soil was applied, but shoot dry weights were statistically similar when 50 µg phosphorus/g soil was applied (Fig. V-2a). Shoot dry weights of nonmycorrhizal control plants and plants inoculated with *G. dimorphicum* were statistically similar at all levels of phosphorus applied. In the presence of the pathogen, shoot dry weights of nonmycorrhizal and mycorrhizal plants were similar when 0 and 10 µg phosphorus/g soil were applied (Fig. V-2b). When 20 µg phosphorus/g soil was applied, shoot dry weight of plants colonized by *G. intraradices* were significantly higher than nonmycorrhizal control plants but similar to plants colonized by *G. dimorphicum* and *G. mosseae*. When 50 µg phosphorus/g soil was applied, plants colonized by *G. intraradices* and *G. mosseae* had significantly higher shoot weight than nonmycorrhizal control plants but their shoot dry weights were similar to plants colonized by *G. dimorphicum* (Fig. V-2b). The presence of the pathogen in the soil (Fig. V-2b) had little effect in reducing the shoot dry weight when compared to plants not inoculated with the pathogen (Fig. V-2a).

The concentration of phosphorus in the shoots increased as the amount of phosphorus applied to the plants increased (Fig. V-3). In the absence of the pathogen, concentration of phosphorus in the shoots in nonmycorrhizal and mycorrhizal plants were not significantly different from one another, at all levels of phosphorus applied (Fig. V-3a). In the presence of the pathogen, the concentration

of phosphorus in shoots was not significantly different between nonmycorrhizal and mycorrhizal plants when 0 and 10 μg phosphorus/g soil was applied (Fig. V-3b). However, when 20 μg phosphorus/g soil was applied, the concentration of phosphorus in shoots in all mycorrhizal plants were significantly lower than nonmycorrhizal control plants, with the differences becoming less pronounced and not significantly different when phosphorus levels applied to soil were 50 μg phosphorus/g soil (Fig. V-3b). The presence of *B. sorokiniana* in the soil had no effect on the phosphorus concentration in the shoots (Fig. V-3).

The phosphorus concentration in the roots significantly increased as the application of phosphorus to the plants increased, particularly when 50 μg phosphorus/g soil was applied (Fig. V-4). In the absence of the pathogen, no significant difference in phosphorus concentration in roots between nonmycorrhizal and mycorrhizal plants was observed (Fig. V-4b). The phosphorus concentration in the roots was significantly greater in the mycorrhizal plants than in the nonmycorrhizal plants at 50 μg phosphorus per gram soil in the presence of *B. sorokiniana* (Fig. V-4b). However, when less phosphorus was applied to the soil, no significant difference in phosphorus concentration in roots between nonmycorrhizal and mycorrhizal plants was seen (Fig. V-4b).

VA mycorrhizal root colonization decreased as the concentration of phosphorus applied to the plants increased, both in the absence (Fig. V-5a) and presence (Fig. V-5b) of *B. sorokiniana*. The highest level of VA mycorrhizal root colonization occurred when no phosphorus was applied to the plants, with a dramatic decrease in VA mycorrhizal root colonization occurring with any application of phosphorus to the soil. The levels of root colonization by all three *Glomus* species were similar when 10, 20, and 50 μg phosphorus/g soil were applied (Fig. V-5). In addition, the level of root colonization by all three *Glomus* species was not affected by the presence of the common root rot pathogen.

D. Discussion

The application of phosphorus to soil reduced common root rot severity but the addition of VA mycorrhizal fungi with phosphorus significantly reduced the severity of disease even further. Furthermore, all three *Glomus* species were able to suppress the disease at all levels of phosphorus applied. Other studies reported a reduction in incidence of barley common root rot disease by VA mycorrhizal fungi (Grey *et al.*, 1989; Rempel and Bernier, 1990). Grey *et al.* (1989) showed that the

application of phosphorus alone did not significantly reduce disease. Piening *et al.* (1983) also observed that the addition of phosphorus to fallow land did not significantly reduce the incidence of root rot. However, Verma *et al.* (1975) observed a less rapid development of root rot in wheat grown in soils high in phosphorus. It is therefore unclear whether additional soil phosphorus has any suppressing effect on common root rot disease. It has been suggested that these conflicting results may be due, in part, to differences in cultivar reactions to common root rot as well as the time when pathogen infection occurs in the plant root (Piening *et al.*, 1983).

Glomus dimorphicum was less effective at reducing disease severity than *G. intraradices* and *G. mosseae* . Effectiveness of various VA mycorrhizal species has been demonstrated at different levels of soil phosphorus. Some species can operate over a wide range of phosphorus levels while others are only effective at low levels of phosphorus (Schubert and Hayman, 1986). It has also been demonstrated that VA mycorrhizal effectiveness can vary among VA mycorrhizal fungal species (Boyetchko and Tewari, 1990) and the present study further substantiates these results.

Application of phosphorus to the plants led to increases in shoot dry weights of mycorrhizal plants and nonmycorrhizal plants. When no phosphorus was applied to the soil, mycorrhizal and nonmycorrhizal plants had similar shoot dry weights. As the phosphorus levels applied to the soil increased, significant differences in shoot dry weights between nonmycorrhizal and mycorrhizal plants, particularly plants inoculated with *G. intraradices* and *G. mosseae*, were observed. Grey *et al.* (1989) observed similar results and suggested that a minimum level of phosphorus is required to obtain an improvement in plant growth. The phosphorus concentration in shoots of mycorrhizal plants was lower than that in nonmycorrhizal plants when 50 μg phosphorus/g soil was applied, but phosphorus concentrations were essentially the same in mycorrhizal and nonmycorrhizal plants when no phosphorus was applied. Higher levels of phosphorus applied to the soil had a negative effect on VA mycorrhizal fungi. This was reflected by a dramatic decrease in root colonization by all three *Glomus* species. The negative effect of applied phosphorus on VA mycorrhizal fungi may have led to reduced phosphorus uptake by the fungi and, hence, lower shoot phosphorus concentrations. Davis and Menge (1980) also reported a loss of beneficial effect in mycorrhizal citrus plants fertilized with more than 56 μg phosphorus/g soil.

Phosphorus concentrations in the roots of mycorrhizal plants were similar to nonmycorrhizal plants at 50 µg phosphorus/g soil, in the absence of the pathogen. However, in the presence of *B. sorokiniana*, nonmycorrhizal plants had significantly lower concentrations of phosphorus in the roots than mycorrhizal plants. Root phosphorus concentrations were also lowest in peanut plants infected with *Sclerotium rolfsii* Sacc. alone than in peanut plants with mycorrhizal fungi alone (Krishna and Bagyaraj, 1983). Their results, though, were based on only one level of phosphorus application. The results from the present study suggests that there may be an effect of *B. sorokiniana* on phosphorus because phosphorus concentrations in roots were higher in mycorrhizal plants than nonmycorrhizal plants in the presence of the pathogen. However, phosphorus alone is not playing a major role in suppression of common root rot disease. This is further demonstrated by the lack of any dramatic reduction in disease severity by the addition of phosphorus alone. The results indicate that resistance of mycorrhizal barley plants to *B. sorokiniana* may also be attributed to factors other than just the improved phosphorus content in the roots.

Root colonization by all three *Glomus* species was not affected by the presence of *B. sorokiniana* . Other studies have reported increases in VA mycorrhizal root colonization in presence of the pathogen (Kaye *et al.*, 1984; Caron *et al.*, 1986a) but no explanation was offered for the results. Our results suggest that the presence of *B. sorokiniana* has no effect on the competition with VA mycorrhizal fungi for infection sites on barley roots. Increasing phosphorus levels led to dramatic decreases in root colonization by all three VA mycorrhizal species. However, even when mycorrhizal root colonization was reduced, the ability of all three *Glomus* species to suppress disease was not significantly affected. The results indicate that disease suppression is, therefore, not a result of improved uptake of phosphorus alone by the VA mycorrhizal fungi tested.

It appears that the mechanism of biological control of barley common root rot, caused by *B. sorokiniana* , by VA mycorrhizal fungi is multicomponent and the effect of phosphorus is only one of several mechanisms involved in suppression of the disease. Further investigations on the mechanism(s) involved in biological control of common root rot disease are being conducted. Other researchers believe that alterations in host physiology induced by VA mycorrhizal fungi affect plant pathogens. Mycorrhizal roots may be more lignified (Dehne, 1982), and contain higher levels of arginine (Baltruschat and Schönbeck, 1975), or phenolics (Krishna and Bagyaraj, 1983) which inhibit pathogens. The production

of antimicrobial compounds by VA mycorrhizal fungi may also improve the resistance of plants to soil-borne pathogens (Duchesne *et al.*, 1987). The current study has also indicated the need to study more than one species of VA mycorrhizal fungi since differences in the effectiveness of each species may occur. Similarly, a number of cultivars may respond in different ways to VA mycorrhizal fungi (Azcon and Ocampo, 1981; Boyetchko and Tewari, 1990). Therefore, it is possible that more than one mechanism may be involved in the suppression of soil-borne diseases by VA mycorrhizal fungi. It would be simplistic to believe that phosphorus nutrition is the sole mechanism for disease reduction by these fungi.

E. Figures and Legends

Figure V-1. Effect of applied phosphorus on the disease rating (%) in barley inoculated *G. dimorphicum* (G. dim), *G. intraradices* (G. intr), or *G. mosseae* (G. mos) without (C-) or with (C+) *B. sorokiniana*. Controls consisted of plants without mycorrhizal fungi. Bar on graph represents LSD at P = 0.05.

Figure V-1

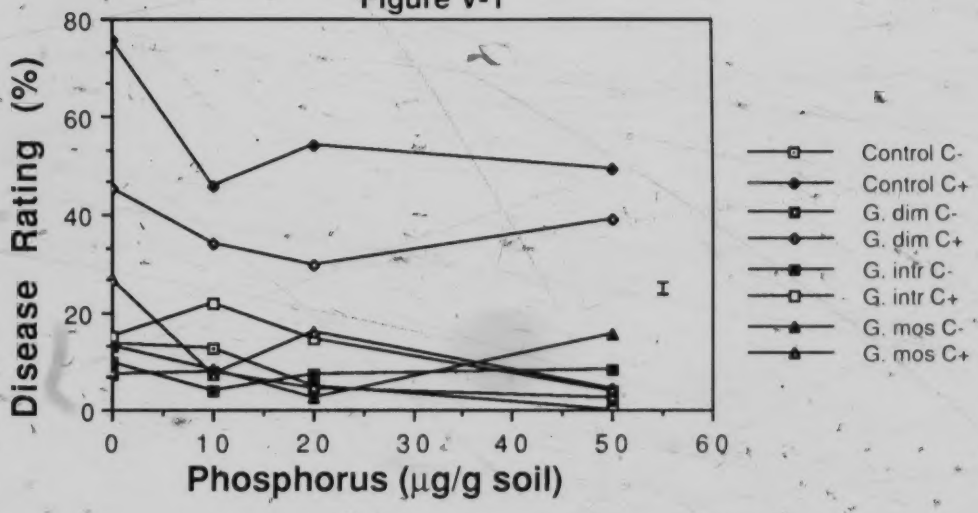
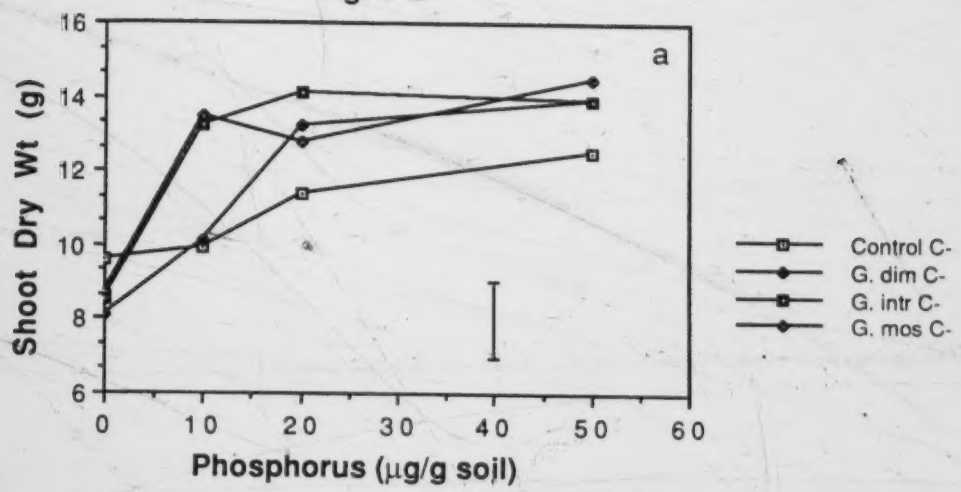

Disease Rating (%)
80
60
40
20
0
0
10
20
30
40
50
60
Phosphorus (µg/g soil)
Control C-
Control C+
G. dim C-
G. dim C+
G. intr C-
G. intr C+
G. mos C-
G. mos C+

Figure V-2. Effect of applied phosphorus on barley shoot dry weight (based on ten plants per sample) inoculated with *G. dimorphicum* (G. dim), *G. intraradices* (G. intr), or *G. mosseae* (G. mos) without (C-) (Fig. V-2a) or with (C+) (Fig. V-2b) *B. sorokiniana*. Controls consisted of plants without mycorrhizal fungi. Bars on graphs represent LSD at P = 0.05.

Fig. V-2


a
Shoot Dry Wt (g)
16
14
12
10
8
6
0
10
20
30
40
50
60
Phosphorus (µg/g soil)
Control C-
G. dim C-
G. intr C-
G. mos C-

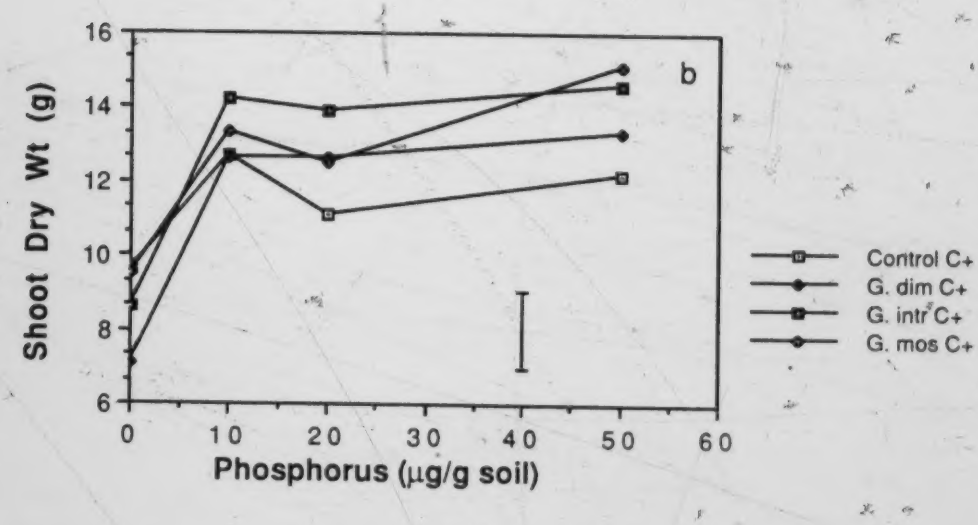

b
Shoot Dry Wt (g)
16
14
12
10
8
6
0
10
20
30
40
50
60
Phosphorus (µg/g soil)
Control C+
G. dim C+
G. intr C+
G. mos C+

Figure V-3. Effect of applied phosphorus on concentration of phosphorus in barley shoots inoculated with *G. dimorphicum* (G. dim), *G. intraradices* (G. intr), or *G. mosseae* (G. mos) without (C-) (Fig. V-3a) or with (C+) (Fig.V-3b) *B. sorokiniana*. Controls consisted of plants without mycorrhizal fungi. Bars on graphs represent LSD at P = 0.05.

Fig. V-3

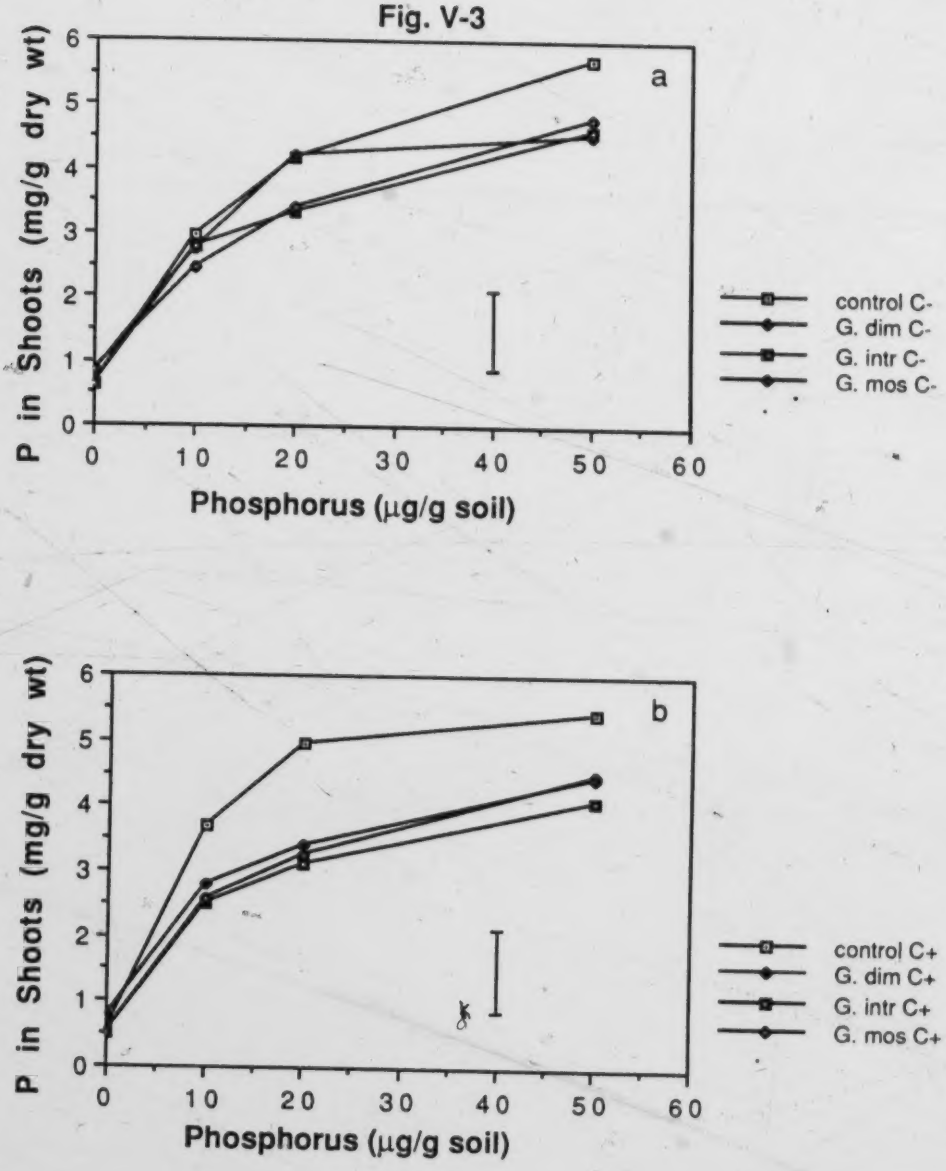

a
P in Shoots (mg/g dry wt)
Phosphorus (µg/g soil)
control C-
G. dim C-
G. intr C-
G. mos C-
b
P in Shoots (mg/g dry wt)
Phosphorus (µg/g soil)
control C+
G. dim C+
G. intr C+
G. mos C+

Figure V-4. Effect of applied phosphorus on concentration of phosphorus in barley roots inoculated with *G. dimorphicum* (G. dim), *G. intraradices* (G. intr), or *G. mosseae* (G. mos) without (C-) (Fig. V-4a) or with (C+) (Fig. V-4b) *B. sorokiniana*. Controls consisted of plants without mycorrhizal fungi. Bars on graphs represent LSD at P = 0.05.

Fig. V-4

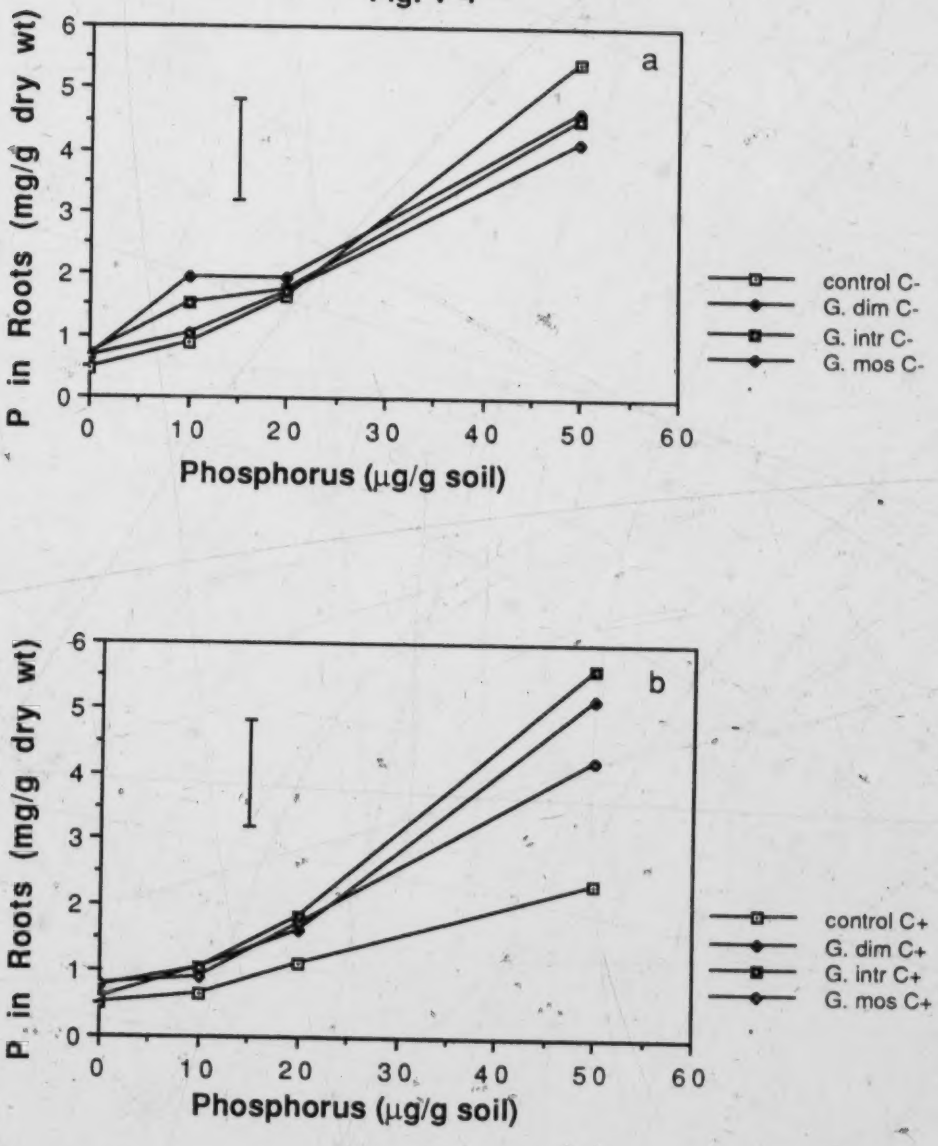
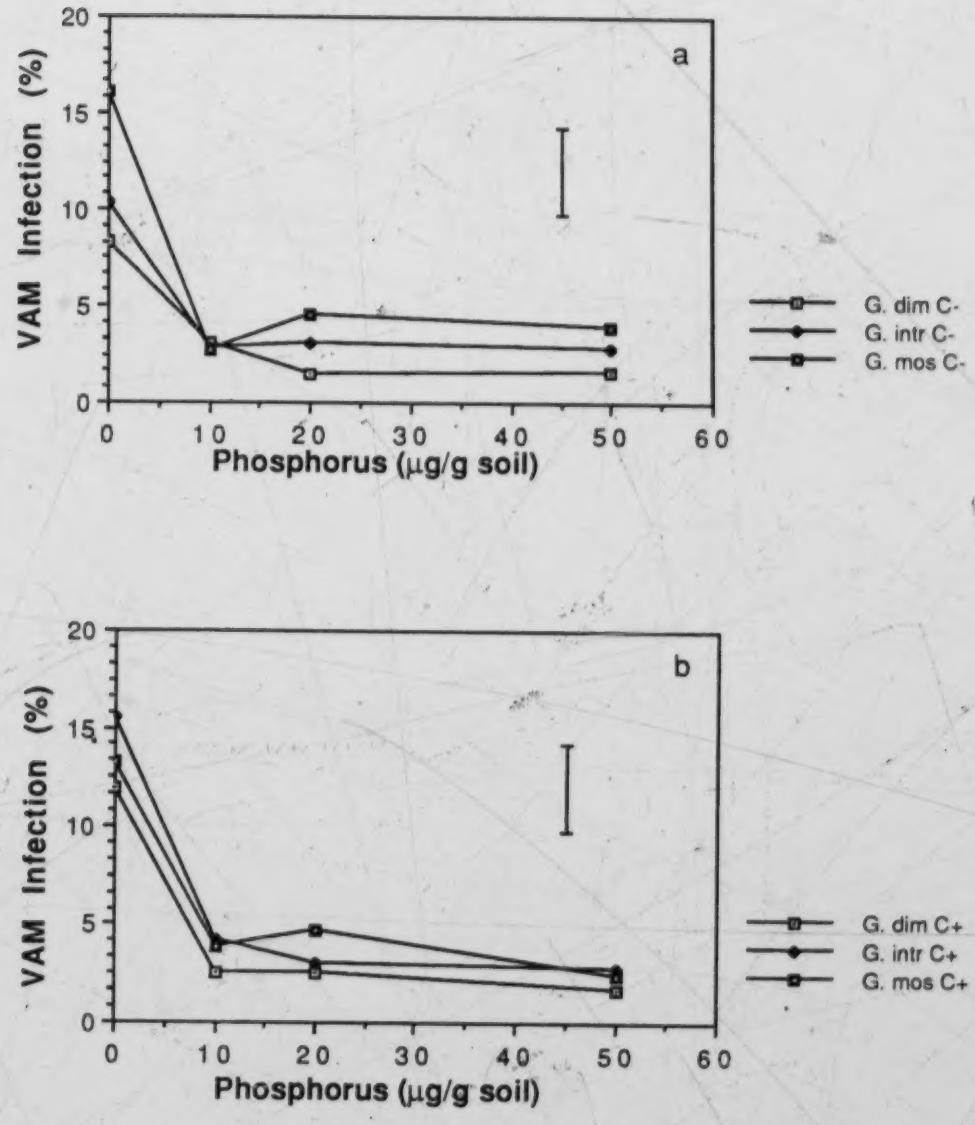

a
P in Roots (mg/g dry wt)
Phosphorus (µg/g soil)
control C-
G. dim C-
G. intr C-
G. mos C-
b
P in Roots (mg/g dry wt)
Phosphorus (µg/g soil)
control C+
G. dim C+
G. intr C+
G. mos C+

Figure V-5. Effect of applied phosphorus on VA mycorrhizal root colonization (%) by *G. dimorphicum* (G. dim), *G. intraradices* (G. intr), or *G. mosseae* (G. mos) either in the absence (C-) (Fig. V-5a) or presence (C+) (Fig. V-5b) of *B. sorokiniana*. All control plants (not inoculated with mycorrhizal fungi) had no VA mycorrhizal root colonization and are thus not included in Figs. V-5a and V-5b. Bars on the graphs represent LSD at P = 0.05.

Fig. V- 5


a
VAM Infection (%)
20
15
10
5
0
0
10
20
30
40
50
60
Phosphorus (µg/g soil)
G. dim C-
G. intr C-
G. mos C-
b
VAM Infection (%)
20
15
10
5
0
0
10
20
30
40
50
60
Phosphorus (µg/g soil)
G. dim C+
G. intr C+
G. mos C+

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Chapter VI

Phytoalexin Elicitation in Barley Roots Infected with *Bipolaris sorokiniana* and VA Mycorrhizal Fungi

A. Introduction

Phytoalexins are compounds produced under various stress conditions and have been given wide attention in the area of disease resistance by plant pathologists (Purkayastha, 1985; Kuc, 1987; Conn and Tewari, 1991). Many phytoalexins (over one hundred) have been isolated and identified from at least 17 families of plants (Kuc, 1987). Paxton (1981) defined phytoalexins as "low molecular weight, antimicrobial compounds that are both synthesized by and accumulated in plants after exposure to microorganisms". It is the rapidity and magnitude of phytoalexin production which determines the degree of disease resistance in the plant (Kuc, 1987; Conn and Tewari, 1991). In cereals, elicitation of phytoalexins has been reported in barley (Oku *et al.*, 1975), oats (Mayama *et al.*, 1981), corn (Leath *et al.*, 1970; Lim *et al.*, 1970), and rice (Ohata and Kozaka, 1969; Triveda and Shinha, 1978; Zuber and Manibhushanrao, 1979). Thus far, the role of phytoalexins in resistance to fungal pathogens in the Poaceae has not been extensively investigated. Most research has concentrated on the phytoalexins produced in the Fabaceae and Solanaceae (Purkayastha, 1985; Kuc, 1987).

Bipolaris sorokiniana (Sacc.) Shoem. causes common root rot disease of barley (*Hordeum vulgare* L.). This disease is economically important throughout the Canadian prairies and can cause yield losses averaging 10.3% annually (Piening *et al.*, 1976). Cultivars of barley vary in their resistance and/or susceptibility to *B. sorokiniana* but very little is known about the precise mechanism of resistance (Anonymous, 1985). Oku *et al.* (1975) provided evidence that phytoalexins are produced in barley leaves in response to infection by *Erysiphe graminis* f.sp. *hordei* Marchal. Compounds known as hordatines have been isolated from barley seedlings grown in the absence of light (Stoessl and Unwin, 1970). These compounds are not considered to be phytoalexins, though, because they are preformed compounds and are not synthesized after exposure to microorganisms. Hordatines have antifungal properties and resistance of barley shoots to *B. sorokiniana* has been correlated with the presence of these compounds (Ludwig *et al.*, 1960; Stoessl and Unwin, 1970). However, there have been no previous

reports on the production of phytoalexins or other antifungal compounds in barley roots.

VA mycorrhizal fungi are known to provide biological control against certain soil-borne plant pathogens (Schenck and Kellam, 1978; Schenck, 1981; Dehne, 1982; Smith, 1988). Barley common root rot severity is reduced in the presence of these symbiotic fungi (Boyetchko and Tewari, 1988; Grey *et al.*, 1989; Thompson and Wildermuth, 1989; Rempel and Bernier, 1990) (see Chapters IV and V). It has also been observed that VA mycorrhizal fungi elicit phytoalexins in soybean roots (Morandi *et al.*, 1984; Morandi and Gianinazzi-Pearson, 1986). The authors suggested that increased plant resistance to soil-borne pathogens may be attributed to elicitation of phytoalexins by VA mycorrhizal fungi contained in plant roots. It was further postulated that an incompatible interaction between an ectomycorrhizal fungus and a host may lead to greater accumulation of phytoalexin (Duchesne *et al.*, 1987). If such is the case, then the inoculation of a host plant with a less compatible VA mycorrhizal fungal species may also result in the elicitation of phytoalexins in plant roots and, thus, provide protection of the host against root pathogenic fungi.

The objective of this study was to determine if phytoalexin elicitation occurs in barley roots when challenged by *B. sorokiniana* and whether barley cultivars of different resistance reactions to *B. sorokiniana* vary in the level of phytoalexin elicitation. It was also the intent of this study to determine if VA mycorrhizal fungi mediate the production of phytoalexins in barley roots which contribute to the resistance of barley to common root rot disease.

B. Materials and Methods

Cultures of *B. sorokiniana* were grown on potato-dextrose agar (PDA) for 10 days at 22 C. Conidia were washed off the plates with distilled water and passed through a double layer of cheese cloth. The resulting conidial suspension was used to inoculate soil.

Barley cultivars used in this study were selected on the basis of their resistance or susceptibility to *B. sorokiniana*. Bonanza and Samson are moderately resistant and Olli and Galt are susceptible. An initial greenhouse experiment was conducted in which plants were inoculated with *B. sorokiniana* to determine their disease ratings. The results from this experiment provided the basis for comparison of phytoalexin production in each cultivar. The barley seed was

surface sterilized for 10 minutes in 3% sodium hypochlorite and placed in a soil mixture (3 sand:1 soil) at a depth of 6 cm in 15 cm pots. The seeds were then covered with 500 cm^3 of the same soil mixture containing either 0 or 4 conidia /cm^3 soil. Initially, 15 seeds were sown and subsequently were thinned to 10 plants per pot. After 8 weeks, the roots were washed free of soil and the subcrown internodes visually examined for discoloration and the disease rating determined according to Ledingham *et al.* (1973) (see Chapter IV).

To screen for the production of phytoalexins, two discs of PDA containing ten-day old cultures of *B. sorokiniana* were placed onto PDA plates. Twenty seeds of each barley cultivar were randomly placed onto the PDA plates containing the pathogen. The controls consisted of the same number of seeds of each cultivar being placed onto PDA plates not containing the pathogen. Each treatment consisted of 10 plates which were incubated for 7 days at room temperature under continuous cool white fluorescent light which provided a light intensity of 5.5 $\mu E/m^2/s$. The experiment was replicated 6 times.

Once infection took place, the roots were collected from the PDA plates and the phytoalexins were extracted in 70% (v/v) aqueous methanol according to the method outlined by Conn *et al.* (1988). The extracts were evaporated to dryness at 40 C using a rotary evaporator, redissolved in chloroform, evaporated to dryness again, and redissolved in methanol. The extracts were subsequently filtered to eliminate any remaining debris, spotted onto thin-layer chromatography (TLC) plates, and developed in chloroform/methanol (95:5 v/v). After developing the TLC plates, a bioassay was conducted to determine which spots had antifungal activity. The bioassay consisted of spraying a conidial suspension of *Cladosporium* species in double strength Czapek Dox broth onto the TLC plates and incubating the plates face-down in the dark in a humid chamber at room temperature to allow the fungus to grow. After 2 to 3 days, the plates were examined for areas of inhibition of fungal growth. The phytoalexins were detected by the absence of growth of *Cladosporium* on the TLC plates. The rest of the TLC plate should be dark due to the growth of the fungus.

To prove that antimicrobial compounds were not produced by *B. sorokiniana* alone, the extraction procedure previously described was carried out on conidia and mycelium of *B. sorokiniana*. Conidia were collected from PDA plates while mycelium was collected from shake cultures in potato-dextrose broth grown over a 4 day period. The bioassay previously carried out on extracts from

B. sorokiniana infected barley roots was also carried out on extracts from the conidia and mycelium of the pathogen and results were compared.

To test for antifungal activity against *B. sorokiniana*, the material obtained from the extraction procedure for the pathogen-infected roots was added to a metal cylinder positioned in the centre of a PDA plate previously inoculated with *B. sorokiniana* by spreading the conidia with an inoculation loop across the plate. The extracts from the healthy control plants were used for comparison and the presence or absence of a zone of inhibition where the phytoalexin-containing extract was placed was observed.

To test for elicitation of phytoalexins by VA mycorrhizal fungi, barley cultivar Bonanza was inoculated with soil containing the equivalent of 400 spores of *Glomus dimorphicum* Boyetchko & Tewari, *G. intraradices* Schenck & Smith, and *G. mosseae* (Nicol. & Gerd. & Trappe) per pot and grown in the greenhouse. Cultivar Bonanza was selected as the test plant because more phytoalexin was elicited from this cultivar than from Samson, Galt and Olli. Furthermore, a previous study revealed that Bonanza showed a range of VA mycorrhizal root colonization levels (see Chapter III) which may possibly reflect a range of responses for phytoalexin elicitation. Fifteen seeds were sown in 15 cm pots containing a soil mixture of autoclaved sand and plants were thinned to 10 plants per pot. A control in which no VA mycorrhizal spores were added was used for comparison. Six replicates were included in the experiment. The plants were fertilized weekly with 100 ml Hoagland's solution, minus phosphorus, in addition to regular daily watering. Greenhouse conditions consisted of day/night temperatures of 25-27 C/20 C, a photoperiod of 16 hours and a maximum light intensity of 500 $\mu E/m^2/s$. After 6 weeks, the plants were harvested, roots were washed, and a subsample of each was retained for assessment of VA mycorrhizal root colonization. Extraction for phytoalexins was conducted on the roots of ten-plant samples for each replicate in the same manner as described for the *B. sorokiniana* -infected barley roots. The bioassay using *Cladosporium* was also used to detect the presence of antifungal compounds (i.e. phytoalexins) in the roots.

Assessment for VA mycorrhizal root colonization was conducted by clearing the roots in 10% KOH, and staining in trypan blue-lactic acid staining solution (Phillips and Hayman, 1970). The roots were then placed onto microscope slides and mycorrhizal root colonization assessed using the grid-line intersect method (Giovannetti and Mosse, 1980) (see Chapter III).

C. Results

The disease ratings of the barley cultivars revealed that Olli was the most susceptible cultivar to the isolate of *B. sorokiniana* used with a disease rating of 54.5 (Table VI-1). Olli was statistically similar to Galt in disease susceptibility, which had a disease rating of 39.0, but Bonanza and Samson had significantly lower disease ratings of 26.3 and 21.3, respectively, than Olli (Table VI-1). In the controls, where no conidia of *B. sorokiniana* were added to the soil, some disease was observed on the subcrown internodes in all the barley cultivars except Bonanza (Table VI-1). The disease levels in the uninoculated roots of Galt and Olli were statistically similar to those of the pathogen-infected Bonanza and Samson roots (Table VI-1). The control plants of Bonanza, Samson, and Galt had statistically similar levels of disease in the roots (Table VI-1). The disease in control plants may be the result of the presence of seed-borne inoculum of the pathogen. Seed surface-sterilization was, therefore, not completely effective at eliminating the pathogen from the seed.

The TLC bioassay revealed that all 4 barley cultivars, when infected with *B. sorokiniana* , elicited phytoalexin, while control plants did not elicit these compounds (Fig. VI-1). One type of phytoalexin was produced, in all pathogen-infected cultivars, with an Rf value of 0.70. Bonanza exhibited more phytoalexin production than the other three cultivars (Fig. VI-1).

The extraction procedure conducted on conidia and mycelium of *B. sorokiniana* revealed the presence of an antifungal compound from the conidia but not from the mycelium (Fig. VI-2). The compound extracted had an Rf value of 0.78 which was different than the phytoalexin elicited in the barley roots (Fig. VI-2). Therefore, the compound extracted from the conidia of *B. sorokiniana* was a different compound than that extracted from the barley roots infected with the pathogen.

The assay to test for antifungal activity against *B. sorokiniana* showed that the extract from Bonanza roots infected with the pathogen was inhibitory while the extracts from the healthy control plant roots was not inhibitory to *B. sorokiniana* (Fig. VI-3). Therefore, the phytoalexin produced, in response to infection by *B. sorokiniana* , was not only inhibitory to *Cladosporium* species, as observed in the TLC bioassay, but it was also inhibitory to the pathogen causing barley common root rot disease.

The TLC bioassay for phytoalexin elicitation in the barley roots of Bonanza, colonized by all three *Glomus* species, showed no phytoalexin activity (Fig. VI-4). Also, control plants showed no elicitation of phytoalexin (Fig. VI-4). Examination of the barley roots for VA mycorrhizal colonization revealed the presence of VA mycorrhizal fungi in the roots inoculated with all three *Glomus* species, although root colonization levels were low in all cases (Table VI-2). No VA mycorrhizal fungal structures were observed in the nonmycorrhizal control plants (Table VI-2).

D. Discussion

Very little information is available on the role that phytoalexins play in disease resistance in the cereal crops (Leath *et al.*, 1970; Lim *et al.*, 1970; Mayama *et al.*, 1981; Oku *et al.*, 1975). Most reports have considered the elicitation of phytoalexins in the shoot portion of the plant but this paper reports on the first investigation of phytoalexin accumulation in roots of barley when infected with *B. sorokiniana.*

More phytoalexin was produced in the roots of cultivar Bonanza than the other three cultivars, which produced similar amounts of phytoalexin. It has been suggested that an incompatible reaction with a pathogen would result in a greater level of phytoalexin produced (Mayama *et al.*, 1981). Results from this study generally support this since Bonanza was shown to be one of the most resistant cultivars, of the four cultivars tested, to *B. sorokiniana.* However, the reason for the lower amount of phytoalexin accumulation in roots of Samson, another moderately resistant cultivar, is yet unclear. Since a time course study was not conducted in our experiments, it would be interesting to determine the rapidity of phytoalexin production in each barley cultivar and to correlate the speed of phytoalexin accumulation in roots to more resistant cultivars. Conn *et al.* (1988) showed that crucifers which are less susceptible to *Alternaria brassicae* (Berk.) Sacc., the causal agent of blackspot disease of rapeseed, produced more phytoalexins than crucifers which are more susceptible to the pathogen. Lim *et al.* (1970) revealed that different isolates of *Helminthosporium turcicum* Pass. showed variation in the degree of phytoalexin elicitation in corn. A more pathogenic isolate induced the production of greater amounts of phytoalexins than weakly pathogenic isolates. However, the authors did not completely rule out the possibility that the antimicrobial compounds were produced also by the pathogen. Droplets from leaves, which contained spores of *H. turcicum,* were used to isolate

phytoalexins, after spores and germ tubes of the pathogen were removed from the droplets by centrifugation. Our study showed that an antimicrobial compound was extracted from the conidia of *B. sorokiniana* but not from the mycelium. This antimicrobial compound cannot be classified as a phytoalexin because the working definition indicates that phytoalexins are synthesized in plants after exposure to microorganisms (Paxton, 1981). Since the conidia are highly pigmented, as revealed by their dark color, it is possible that phenolic compounds were extracted from the conidia which may be inhibitory to fungal growth. However, the Rf value of the antimicrobial compound extracted from the conidia of *B. sorokiniana* was different from the Rf value of the phytoalexin extracted from infected barley roots. This indicated that the two compounds are different, although they both inhibited the growth of *Cladosporium* in the TLC bioassay. Furthermore, the roots themselves are colonized by the hyphae (mycelium) of *B. sorokiniana* , as opposed to conidia. No antifungal compound was extracted from the mycelium of *B. sorokiniana* which also indicated that phytoalexins were elicited by plant roots when challenged by the pathogen. It would, however, be interesting to compare the effect of various isolates of *B. sorokiniana* on the elicitation of phytoalexins, particular in relation to the quantity and speed of phytoalexin accumulation.

The nature of the phytoalexin extracted from the barley roots is unknown. Antifungal factors have been previously isolated from barley shoots (Koshimizu *et al.*, 1963; Stoessl and Unwin, 1970). These compounds, known collectively as hordatines, are produced in senescing tissue (Ludwig *et al.*, 1960). The activity of hordatines in barley seedlings decreased with age and divalent cations, such as calcium, were linked to decrease in their activity. The susceptibility of barley shoots to *Helminthosporium sativum* Pamm., King & Bakke (=*B. sorokiniana*) was also associated with senescence of plant tissue and, thus, a decrease in hordatine activity (Ludwig *et al.*, 1960; Stoessl and Unwin, 1970). It is clear, from our study that the phytoalexin extracted from barley roots infected with *B. sorokiniana* is not one of the hordatines discussed by Stoessl and Unwin (1970). The authors showed the Rf value of one of the three hordatines to be 0.70, using a developing solvent of n-butanol-water-acetic acid (4:5:1). The procedure used in our study revealed that the phytoalexin elicited in barley roots also had an Rf value of 0.70, but the developing solvent consisted of chloroform-methanol (95:5). Furthermore, hordatines are preformed compounds found in senescing tissue while phytoalexins are synthesized by plants after being exposed to microorganisms (Paxton, 1981). In our study, the control plants contained no antifungal

compounds but these compounds were found in plants which were challenged by *B. sorokiniana*.

The phytoalexin extracted from barley roots infected by the pathogen had antimicrobial properties against *B. sorokiniana* , the same pathogen causing the elicitation of the phytoalexin. The TLC bioassay also showed the phytoalexin to be inhibitory to *Cladosporium*. A more extensive study may reveal that this compound is inhibitory to other plant pathogens also since the accumulation of phytoalexins is specific to the host and not the pathogen (Purkayastha, 1985).

The TLC bioassay for phytoalexin elicitation by VA mycorrhizal fungi revealed that no antimicrobial compounds were produced by barley against any of the *Glomus* species tested. Although Morandi *et al.* (1984) observed the accumulation of three phytoalexins by *G. mosseae* and *G. fasciculatum* in soybean roots, the quantities detected were very small. After 8 weeks, 0.14 µg glyceollin, 24.9 µg coumestrol and 13 µg daidzein, per gram fresh root weight, were detected in roots colonized by both *G. mosseae* and *G. fasciculatum* in the same root. However, coumestrol and daidzein were not inhibitory to fungi (Morandi *et al.*, 1984). It is possible that the TLC cladosporium bioassay to test for phytoalexin elicitation by VA mycorrhizal fungi was not sensitive enough to detect small amounts of phytoalexin produced. Further purification and detection of compounds by more sensitive techniques such as HPLC is required. Until then, it is not ruled out that phytoalexins may be accumulating in mycorrhizal barley roots in response to VA mycorrhizal fungi which may be playing a role in the suppression of barley common root rot disease. It is possible that mycorrhizal fungi could be used to immunize (Conn and Tewari, 1991) host plants against root pathogens but further research is required to examine the role of phytoalexins in mycorrhizal symbioses.

E. Tables, Figures, and Legends

Table VI-1. Disease Ratings for Common Root Rot Disease

Cultivar	*Bipolaris sorokiniana* (Conidia/cm^3 soil)	
	0	4
Bonanza	0.0 e	26.3 bc
Samson	8.0 de	21.3 c
Galt	16.0 cde	39.0 ab
Olli	18.0 cd	54.5 a
LSD	16.5	

Numbers followed by the same letter are not significantly different from one another using LSD at P=0.05.

Table VI-2. VA Mycorrhizal Root Colonization (%) in Barley Cultivar Bonanza

VA Mycorrhizal Species	Colonization (%)
control	0.00 b
G. dimorphicum	7.59 a
G. intraradices	11.66 a
G. mosseae	6.63 a
LSD	5.07

Numbers followed by the same letter are not significantly different from one another using LSD at P=0.05

Figure VI-1. TLC cladosporium bioassay for phytoalexins with extracts from roots of cultivar Bonanza (A,B), Samson (C,D), Galt (E,F), and Olli (G,H). Extracts from healthy roots are in lanes A,C,D,G while those from roots infected with *B. sorokiniana* are in lanes B,D,F,H. Clear areas (arrowheads) indicate areas of inhibition where *Cladosporium* did not grow, which indicates the presence of phytoalexins. Dark areas indicate the growth of *Cladosporium* . All controls (A,C,E,G) were negative.

A
B
C
D
E
F
G
H

Figure VI-2. TLC cladosporium bioassay for phytoalexins. Comparison of extracts from healthy roots of Bonanza (A), Bonanza roots infected with *B. sorokiniana* (B), Conidia (C), and mycelium (D) of *B. sorokiniana* alone. Presence of phytoalexin in infected roots is indicated by an arrowhead. Note the presence of an antifungal compound from extracts of *B. sorokiniana* conidia (arrow) (C) while extracts from mycelium show no such compound (D).

A
B
C
D

Figure VI-3. Assay for antifungal activity against *B. sorokiniana* . The area of PDA plate where extract from Bonanza healthy roots was placed (A) shows growth of *B. sorokiniana* . The area of PDA plate where extracts from Bonanza roots infected with *B. sorokiniana* (B) was placed shows an area of inhibition which prevented growth of the pathogen.

a
b

Figure VI-4. TLC cladosporium bioassay for phytoalexins. Extracts from roots of cultivar Bonanza which had no *Glomus* species (A), *G. intraradices* (B), and *G. mosseae* (C), and *G. dimorphicum* (D) in their roots. Note the lack of growth inhibition of *Cladosporium* in all cases, as seen by dark growth of the fungus in all the lanes (A,B,C,D).

A
B
C
D

F. References

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Chapter VII

Quantitative Comparison of Root Exudates of Four Barley Cultivars

A. Introduction

The rhizosphere effect refers to the area of intense microbial activity around the roots of plants growing in soil (Rovira, 1956; Ayers and Thornton, 1968). Root exudates are comprised of compounds which leak from roots into the rhizosphere and may represent a significant loss of plant photosynthate (Rovira, 1969). These root exudates may stimulate the activity of organisms which suggests that soil organisms are capable of using components in the exudates (Rovira, 1956). In phosphorus deficient soils, a correlation was observed between increased root exudation and reduced root phospholipid levels, which are associated with changes in root membrane permeability (Ratnayake *et al.*, 1978; Graham *et al.*, 1981).

Application of phosphorus to soil, as well as VA mycorrhizal fungi, which are known to improve plant phosphorus uptake (Hayman, 1983), leads to reduction in root exudation by some plants (Ratnayake *et al.*, 1978; Graham *et al.*, 1981). Rates of root exudation were reportedly lower in plant species which are generally not susceptible to VA mycorrhizal fungi than in plant species which are readily colonized by these fungi (Schwab *et al.*, 1984). While studying different wheat cultivars for their mycorrhizal dependency, Azcon and Ocampo (1981) observed that cultivars with the lowest rate of exudation had the least amount of VA mycorrhizal root colonization. Graham and Menge (1982) showed that severity of take-all disease of wheat, caused by *Gaeumannomyces graminis* (Sacc.) von Arx. and Olivier var. *tritici* Walker, was greater in phosphorus deficient soils and that application of phosphorus to soil resulted in reduced root exudation and thus disease severity. Addition of VA mycorrhizal fungi to soil had a similar effect on disease. The suppression of soil-borne diseases by VA mycorrhizal fungi may, therefore, result through the regulation of root exudation and pathogen activity. It is also possible that cultivars more resistant to plant pathogens may show lower root exudation patterns. Furthermore, regulation of root exudation in plants may affect the resistance or susceptibility of plants to soil-borne pathogens.

The objective of this study was to screen barley cultivars for root exudation levels and to compare them to resistance/susceptibility of the cultivars to *B.*

sorokiniana, as well as to VA mycorrhizal root colonization. The amount of reducing sugars and amino acids in root exudates of four barley cultivars which differ in their susceptibility to VA mycorrhizal fungi and to *B. sorokiniana* (Sacc.) Shoem were compared. To date, it is not known whether root exudation plays a role in the resistance of barley cultivars to common root rot disease.

B. Materials and Methods

Barley cultivars used in this study were chosen on the basis of their resistance or susceptibility to *B. sorokiniana*. A previous experiment, outlined in Chapter VI, was conducted to provide data of disease ratings for Bonanza and Samson, which are moderately resistant, and Galt and Olli, which are susceptible to *B. sorokiniana*.

Root exudates were collected from plants of barley cultivars grown under aseptic conditions. Seeds of each cultivar were sterilized in 3.5% hydrogen peroxide for 1.5 hours in a flask on a shaker, rinsed with sterile distilled water, placed in 2% sodium hypochlorite on a shaker for an additional 2 hours, and rinsed with sterile distilled water 4 to 5 times. The seeds were then germinated on potato-dextrose agar (PDA) plates to test for sterility. After 2 days, germinated seeds, not colonized by bacteria or fungi, were transferred onto plastic mesh screens place in large test tubes containing 25 ml nutrient solution and sealed with a cotton plug to maintain sterile conditions. The nutrient solution consisted of 0.5 ml of a number of stock solutions, each, per 1 litre distilled water. The various stock solutions used to prepare the nutrient solution are listed in Table VII-1. Twelve plants of each cultivar were grown individually in test tubes, placed in the greenhouse, with the nutrient solution being collected and replaced every 3 days. Greenhouse conditions consisted of day/night temperatures of 25-27 C/20 C, a photoperiod of 16 hours and a maximum light intensity of 500 $\mu E/m^2/s$. Each sample consisted of root exudates of 3 plants collected over the 14-day period and thus, 4 replicates for each cultivar. Root exudates collected were concentrated on a rotary evaporator, transferred to small vials, freeze-dried, and stored at -4 C until reducing sugars and amino acids were analyzed.The freeze-dried material was redissolved in 2 ml distilled water, with 1 ml each used for analysis of reducing sugars and amino acids.

The Nelson-Somagyi reducing sugar test was used to determine total reducing sugars in the root exudates (Nelson, 1944; Somogyi, 1952). Each sample

was added to 1 ml copper reagent, boiled in a water bath for 20 minutes, and cooled in ice water. One ml of arseno-molybdate was added directly into the solution, shaken vigorously, and reducing sugar concentration determined colorimetrically when CO_2 evolution ceased (approximately 30 to 60 minutes). Colorimetric analysis, using glucose as a standard, was used to determine total reducing sugars spectrophotometrically at absorbance 500 nm.

Total amino acid concentration, using ninhydrin, was determined on the root exudate samples (Spies, 1957). To each sample, 0.5 ml cyanide-acetate buffer and 0.5 ml 3% ninhydryin were added. After heating for 15 minutes in a boiling water bath, samples were placed in an ice-water bath, and 5 ml of 50% ethanol was added. Colorimetric analysis, using leucine as a standard, was used to determine total amino acids spectrophotometrically at Absorbance 570 nm.

The concentration of reducing sugars and amino acids from root exudates of the four barley cultivars were compared by regression analysis with disease ratings for common root rot, and VA mycorrhizal root colonization by three different *Glomus* Tul. & Tul. species. The data for disease ratings and VA mycorrhizal colonization have been presented in Chapters VI and III, respectively, and are briefly summarized in Table VII-3 in this chapter.

C. Results

No significant difference in concentration of reducing sugars from root exudates among the four barley cultivars tested was observed. Root exudates from Bonanza and Samson contained 308.7 and 339.2 µg reducing sugars/g dry root weight, respectively, while those from Galt and Olli contained 109.6 and 271.7 µg reducing sugars/g dry root weight, respectively (Table VII-2). Similarly, the amounts of amino acids in the root exudates among the barley cultivars were not significantly different from one another, where root exudates from Bonanza and Samson contained concentrations of amino acids at 189.5 and 134.6 µg/g dry root, respectively. Root exudates from Galt and Olli contained 48.9 and 105.4 µg amino acids/g dry root weight, respectively (Table VII-2).

Although significant differences in reducing sugars and amino acids among the barley cultivars were not observed, regression analyses were conducted to study the relationships of these components of the root exudates to disease ratings and VA mycorrhizal root colonization of the barley cultivars. Data for disease ratings and VA mycorrhizal assessments were given and discussed in detail in Chapters

VI and III. These data are summarized in this chapter in Table VII-3 and are used in the regression analyses. Generally, Bonanza and Samson had lower disease ratings than Galt and Olli, with Olli having the highest disease rating of 54.5 (Table VII-3). The level of VA mycorrhizal root colonization for each barley cultivar varied with the *Glomus* species used. In addition, differences in susceptibility of each barley cultivar to each VA mycorrhizal species tested were observed (Table VII-3).

A negative correlation between amount of reducing sugars and disease rating was seen, with a correlation coefficient of -0.993. However, comparison of the three individual *Glomus* species with concentration of reducing sugars revealed no correlation between VA mycorrhizal root colonization and root exudation. The correlation coefficients were extremely low.

Regression analysis for amino acids revealed a negative correlation with disease rating, with a correlation coefficient of -0.863. However, no correlations were observed when amino acid concentrations in root exudates were compared with the level of VA mycorrhizal root colonization for each *Glomus* species with correlation coefficients being extremely low.

D. Discussion

Comparison of the four barley cultivars, which differ in their susceptibility to barley common root rot disease, showed no significant differences in concentration of reducing sugars and amino acids in their root exudates. This was due largely to the high variability between replicates. These results differ from those reported by Azcon and Ocampo (1981) who observed a difference in total reducing sugars in root exudates among thirteen wheat cultivars. However, the basis for the selection of the thirteen wheat cultivars was not provided, although the cultivars were being tested for mycorrhizal dependency.

In the present study, phosphorus was supplied in the nutrient solution, and it is not known at present whether root exudation patterns may differ among barley cultivars grown under a series of phosphorus regimes. Ratnayake *et al.* (1978) provided evidence which showed that exudation of reducing sugars and amino acids from roots of sorghum and citrus was greater when plants were grown under low phosphorus conditions (0, 6, and 28 ppm phosphorus) than under high phosphorus conditions (56, 228, and 556 ppm phosphorus). Since common root rot severity is greater when barley plants are grown in soils low in available

phosphorus (Verma *et al.*, 1975; Piening *et al.*, 1983), it is possible that the amount of root exudation among the barley cultivars may have been greater if the plants were grown under phosphorus deficient conditions.

Chinn and Ledingham (1961) amended soils with glucose, molasses, or vitamin C and observed that germination of spores of *Helminthosporium sativum* Pamm., King, and Bakke (=*B. sorokiniana*) increased in the presence of molasses and vitamin C but not in the presence of glucose. The reason for the lack of germination of *H. sativum* spores in glucose-amended soils was not revealed, although its presence as a component of root exudates would suggest that glucose could have no effect on the population of this pathogen in the rhizosphere. However, Chinn and Ledingham (1961) determined the influence of the three soil amendments by the buried slide technique, and any influence of root exudates from barley roots was not considered.

No correlations were observed between root exudation of reducing sugars and amino acids, among the four barley cultivars tested, and susceptibility to each VA mycorrhizal fungus. However, Azcon and Ocampo (1981) showed that a direct relationship existed between low VA mycorrhizal root colonization and low exudation rates of reducing sugars in thirteen wheat cultivars. Schwab *et al.* (1984) conducted qualitative and quantitative comparisons of root exudates of mycorrhizal and nonmycorrhizal plant species. The authors showed that at early stages of growth, root exudation rates were higher in non-host plants than in host plants. However, measurements conducted at 3 to 6 weeks after seed germination revealed that root exudation rates were higher in the mycorrhizal host plants. The early period of high root exudation rates in non-host plants (3 weeks or less) coincides with the lag phase when VA mycorrhizal spores germinate and germ tubes grow towards the rhizosphere. After 3 weeks, when root exudation rates in non-host plants decline, the amount of root exudate may not be high enough to sustain or support the VA mycorrhizal fungus (Schwab *et al.*, 1984). It was also observed that root exudates of mycorrhizal host plants contained no specific reducing sugar, amino acid or carboxylic acid which differed from root exudates of nonmycorrhizal host plants (Schwab *et al.*, 1984). Bécard and Piché (1990) found that root exudates from host plants promoted hyphal growth of spores of *Gigaspora margarita* Becker and Hall while exudates from nonhost plants did not. The authors suggested that exudates from nonhost plants lacked a factor which may stimulate VA mycorrhizal hyphal growth. In the present study, it is possible that no differences in root exudation among the barley cultivars were observed because

the root exudates were collected for a period of 14 days after seed germination. If the experiment was conducted over a longer period of time, an opposite effect may have eventually been seen. It is also possible that root exudates from the barley cultivars tested lacked a compound which may promote hyphal growth of VA mycorrhizal spores and, thus, mycorrhizal root colonization. Future work should consider the length of time for collection of root exudates.

Nelson *et al.* (1986) studied the effect of amendation of culture media with sugars on the interaction between *Enterobacter cloacae* (Jordan) Hormaeche & Edwards and *Pythium ultimum* Trow. *Enterobacter cloacae* is a biological control agent of damping-off disease caused by *P. ultimum*. The bacteria attach to *P. ultimum* hyphae and agglutinate cell wall fragments resulting in inhibition of mycelial growth. The authors observed an inhibitory effect of sugars such as D-glucose, D-galactose, and sucrose on *E. cloacae* and, thus, the ability of the bacterium to control damping-off disease. These sugars blocked binding and agglutination of *E. cloacae* to cell walls of *P. ultimum* hyphae while absence of these sugars resulted in an opposite effect. Nelson *et al.* (1986) suggested that one mechanism involved in biological control by various fungi, bacteria, and nematodes could be specific binding to cell walls of the pathogen. They further speculated that the presence of specific sugars on the seed may affect the ability of *E. cloacae*, or other biocontrol agents, to reduce severity of plant diseases. From the results in our study, it is possible that specific sugars exuding from the root into the rhizosphere may saturate binding sites on the roots which are specific for the recognition of VA mycorrhizal fungi but not for *B. sorokiniana*. This may have further implications in the specificity of plants to VA mycorrhizal fungi and the ability of these symbionts to act as biological control agents. This aspect merits further study when considering mechanisms involved in the biological control of soil-borne diseases by VA mycorrhizal fungi.

Comparison of root exudates of barley cultivars, in this study, considered only quantitative amounts of reducing sugars and amino acids where phosphorus was supplied in the nutrient solution. Other workers revealed a higher rate of root exudation when plants were grown in phosphorus-deficient soils, with a reduction in root exudation occurring when phosphorus was applied to the soil (Graham *et al.*, 1981; Schwab *et al.*, 1983). Furthermore, higher phosphorus levels had no significant effect on the relative proportions of specific amino acids or carboxylic acids, but proportions of arabinose and fructose in the reducing sugar fraction changed (Schwab *et al.*, 1983). However, the authors concluded that the exudation

of specific individual components from the root was not a reliable indicator for determination of effect on VA mycorrhizal fungi. Schwab *et al.* (1983) also suggested that all the material from root exudates is probably not available to the mycorrhizal fungus.

Evidently, more research is required to determine if root exudation from the four barley cultivars plays a role in determining susceptibility or resistance to *B. sorokiniana*, causal agent of barley common root rot disease, or to VA mycorrhizal root colonization. Results given in this study present preliminary information on the levels of reducing sugars and amino acids of barley cultivars. Future studies will consider root exudation under various phosphorus regimes in addition to the effect of VA mycorrhizal root colonization on root exudation.

E. Tables

Table VII-1. Stock solutions needed to prepare the nutrient solution used in root exudate study.

Chemical	Concentration
$Ca(NO_3)_2 \cdot 4H_2O$	2 M
$Mg(NO_3)_2 \cdot 6H_2O$	0.6 M
NH_4NO_3	0.6 M
K_2HPO_4	0.2 M
K_2SO_4	0.2 M
KNO_3	0.8 M
$MnCl_2.4H_2O$	4 mM
H_3BO_3	0.12 M
$ZnSO_4.7H_2O$	1 mM
$CuSO_4.5H_2O$	0.3 mM
Na_2MoO_4	0.235 mM
$FeCl_3.6H_2O$	0.02 M
Na_2EDTA	0.02 M

Table VII-2. Concentrations of reducing sugars and amino acids collected from root exudates[a] of barley cultivars grown over a 14-day period.

Cultivar	Reducing sugars[b]	Amino Acids[c]
Bonanza	308.7	189.4
Samson	339.2	134.6
Galt	190.6	48.9
Olli	271.7	105.4
SE	111.1	125.0

a. Reducing sugars and amino acids (means of 4 replicates) are recorded as µg/g dry root weight.

b,c. There was no significant difference in reducing sugars or amino acids as determined by analysis of variance at P = 0.05.

Table VII-3. Summary of data for disease ratings[a] and VA mycorrhizal root colonization (%)[b] by *Glomus dimorphicum*, *G. intraradices*, and *G. mosseae*.

Cultivar	Disease Rating	VA mycorrhizal root colonization (%)		
		G. dimorphicum	*G. intraradices*	*G. mosseae*
Bonanza	26.3	8.0	18.5	4.1
Samson	21.3	7.0	6.9	6.2
Galt	39.0	3.7	6.6	1.8
Olli	54.5	7.8	7.9	5.7

a. Data for disease rating were previously provided in Table VI-1 and discussed in detail in Chapter VI.

b. Data for VA mycorrhizal root colonization of all *Glomus* species were previously provided in Table III-3 and discussed in detail in Chapter III.

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Chapter VIII

General Discussion and Conclusions

A. Taxonomy

Reports on classification and identification of VA mycorrhizal fungi indigenous to soils in western Canada are limited. Morton (1988) stressed that such information is important with respect to knowledge of species diversity in the VA mycorrhizal fungi. The American Phytopathological Society has also indicated that taxonomy of these fungi is a research priority. Precise identification of VA mycorrhizal species used in a study is necessary before meaningful comparisons with other studies can be made. Other information which is particularly lacking is the identification of VA mycorrhizal species associated with agricultural crops.

In this study, five species of VA mycorrhizal fungi associated with field crops in Alberta were identified. *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe and *G. aggregatum* Schenck & Smith emend. Koske have been the most frequently encountered VA mycorrhizal species in Alberta soils (Zak *et al.*, 1982; Danielson *et al.*, 1984). Other species reported here and in previous studies were *G. tenue* (Greenall) Hall and *Entrophospora infrequens* (Hall) Ames & Schneider (Zak *et al.*, 1982) with *G. pansihalos* Berch & Koske being reported for the first time in western Canada in this study. External proliferation, a phenomenon similar to microcyclic sporulation, was observed for the first time in *G. aggregatum*. This condition results in the elimination of the vegetative stage and in greater sporulation. Induction of this type of sporulation may provide a means for mass production of VA mycorrhizal fungi.

Taxonomic studies of VA mycorrhizal fungi are subject to a number of major limitations. The taxonomy of these fungi is based primarily on the morphological features of spores observed by light microscopy, particularly features of the cell wall. Comparisons are made with species descriptions from the literature which are inconsistent or incomplete, in some cases. The publication of manuals to aid in the taxonomy of VA mycorrhizal fungi has facilitated the procedure of identification and classification. Although accessibility to type specimens is limited, the establishment of the International Collection of VA Mycorrhizal Fungi (INVAM) in West Virginia facilitates access.

Quite often, pot cultures of VA mycorrhizal fungi isolated from the field must be established, an undertaking which requires several months. Smith and Schenck (1985) stressed the need for caution when describing VA mycorrhizal species isolated directly from soil. Boyetchko and Tewari (1986) isolated spores of a dimorphic VA mycorrhizal species, *G. dimorphicum* Boyetchko & Tewari, which were initially believed to belong to two species. However, extensive pot culture studies revealed that the two spore types belonged to one species.

Other information based on biochemical and immunological studies are likely to be useful techniques for identification of VA mycorrhizal species (Morton, 1988). However, these techniques are only currently being assessed. The use of scanning electron microscopy (SEM) proved to be a useful technique for studying the morphology of mycorrhizal spore walls. Observation of spore wall ornamentation, such as the hemispherical warts on the second wall of *G. pansihalos* spores, may be facilitated by techniques such as SEM which provide greater resolution than light microscopy. However, SEM facilities are not readily available to all researchers studying taxonomy. Energy dispersive X-ray microanalysis provided useful information on the chemical nature of the spore walls of VA mycorrhizal fungi. This technique may provide valuable information on the elemental composition of different cell wall layers of mycorrhizal fungi. Although X-ray microanalysis revealed no differences among VA mycorrhizal species in concentration of calcium in their spore walls, this technique may be useful for determining elements, such as calcium, present in spore walls which may be necessary for growth and sporulation of VA mycorrhizal fungi. Clear spore wall surfaces can be distinguished from soil particles by the presence of silicon, in addition to aluminum and iron in the soil particles.

B. Susceptibility of barley cultivars

VA mycorrhizal fungi are host non-specific and have an extensive host range. However, the extent and pattern of VA mycorrhizal root colonization may be influenced by the host (Hetrick *et al.*, 1985; Boyetchko and Tewari, 1990). The current study was undertaken to determine the susceptibility of various barley cultivars to three VA mycorrhizal fungi and whether correlations exist between VA mycorrhizal root colonization and growth and yield of barley plants.

The results revealed no significant differences among barley cultivars in susceptibility to indigenous VA mycorrhizal fungi but differences in susceptibility

were observed among eight barley cultivars to three *Glomus* species used in greenhouse studies. It is possible that indigenous VA mycorrhizal fungi are not present in sufficient numbers or are not strategically located below the seed in sufficient numbers due to agricultural practices such as cultivation which disturbs the soil. Another possibility for the low levels of root colonization by indigenous VA mycorrhizal fungi is that pesticides used in agricultural fields may reduce the capability of VA mycorrhizal fungi to colonize plant roots.

The greenhouse studies revealed differences in the ability of each *Glomus* species to colonize the barley cultivars. This study indicated that a certain degree of host-specificity exists in VA mycorrhizal fungi and that the host-mycorrhizal fungus genotypes may influence the effectiveness of the symbiosis. Some other researchers have also observed significant differences among cultivars of various plant species in susceptibility to VA mycorrhizal fungi (Azcon and Ocampo, 1981; Krishna *et al.*, 1985; Heckman and Angle, 1987; Mercy *et al.*, 1990). It is also possible that resistance to VA mycorrhizal root colonization may be genetically regulated (Duc *et al.*, 1989).

Some cultivars, but not all, inoculated with each *Glomus* species experienced significant increases in growth and yield, depending on the *Glomus* species. Therefore, effectiveness of VA mycorrhizal fungi to improve plant growth and yield is different among VA mycorrhizal species. Also, improvement in growth and yield of mycorrhizal plants over nonmycorrhizal plants was not correlated with the level of VA mycorrhizal root colonization. It may be that a minimum level of mycorrhizal root colonization is essential for any benefit to occur in the plant. High levels of root colonization may be a drain on the plant's photosynthate resulting in lower growth and yield of mycorrhizal plants over nonmycorrhizal plants. Extraradical hyphae produced by VA mycorrhizal fungi are probably playing a more significant role in improved plant growth and yield than the intraradical hyphae.

No relationship was observed between susceptibility of barley cultivars to VA mycorrhizal fungi and susceptibility to *Bipolaris sorokiniana* (Sacc.) Shoem. Toth *et al.* (1990) observed a correlation between resistance of plants to VA mycorrhizal fungi and general resistance of plants to several soil-borne pathogens. It is not known whether general resistance to soil-borne organisms is regulated by the same parameters which regulate resistance to VA mycorrhizal fungi, although it is often assumed that phosphorus and root exudation are involved in all systems of regulation. Further research should consider detailed studies on an individual basis.

It may be that recognition sites on the root surface may also play a significant role in determining the susceptibility of plant species and cultivars to VA mycorrhizal fungi, as well as to other soil-borne organisms.

C. Biological control by VA mycorrhizal fungi

The importance of VA mycorrhizal fungi as biological control agents has been established (Schenck, 1981; Dehne, 1982; Hussey and Roncadori, 1982; Smith, 1988). To the list of diseases controlled by VA mycorrhizal fungi can be added barley common root rot disease, caused by *B. sorokiniana*. All three *Glomus* species tested were effective as biocontrol agents. However, *G. intraradices* and *G. mosseae* were more effective than *G. dimorphicum* in reducing disease severity. Other researchers also showed a reduction in barley common root rot disease by VA mycorrhizal fungi (Grey *et al.*, 1989; Rempel and Bernier, 1990). One study was conducted in the greenhouse (Grey *et al.*, 1989) while it is unknown whether the other study was conducted under field or greenhouse conditions (Rempel and Bernier, 1990). However, Wani *et al.* (1991) found no relationship between common root rot incidence and VA mycorrhizal root colonization. They did observe, however, greater barley yields attributed to VA mycorrhizal fungi which was not related to reduced incidence of disease. The authors considered different cropping systems including an agroecological 8 year rotation and continuous grain system and the effect of each cropping system on the interaction between VA mycorrhizal root colonization and barley common root rot. It was suggested that natural conditions represent a complex system and that a holistic approach be used for studying interactions between plants and soil-borne microorganisms (Wani *et al.*, 1991). Results of the present investigation revealed no significant increase in growth and yield by VA mycorrhizal fungi when disease severity was reduced. However, this portion of the study was conducted when available soil phosphorus was low (i.e. 6 ppm). Further work should also take a holistic approach when studying the interaction between VA mycorrhizal fungi and *B. sorokiniana*.

D. Mechanisms involved in suppression of disease

A few mechanisms, possibly involved in the reduction of barley common root rot disease mediated by VA mycorrhizal fungi, were explored. The role of

phosphorus, phytoalexins, and root exudates in disease suppression were examined in detail.

Many studies have reported that improved phosphorus nutrition by VA mycorrhizal fungi is involved in the suppression of sol-borne diseases (Davis and Menge, 1980; Graham and Menge, 1982; Smith *et al.*, 1986; Carling *et al.*, 1989). In the present study, all *Glomus* species were capable of reducing common root rot severity under various phosphorus regimes, although high phosphorus levels in soil reduced VA mycorrhizal root colonization. Phosphorus, alone, did not reduce common root rot severity as well as application of VA mycorrhizal fungi and phosphorus to soil concommitantly. The results suggested that the *Glomus* species examined improved phosphorus nutrition of barley, resulting in suppression of disease. However, increased uptake of phosphorus was not the only mechanism involved in reduction of common root rot.

The role of phytoalexins in suppression of common root rot disease mediated by VA mycorrhizal fungi was also studied. Morandi *et al.* (1984) observed an accumulation of phytoalexins in mycorrhizal soybean roots. One theory suggests that low levels of phytoalexins elicited by these symbionts may immunize plants against soil-borne pathogens (Duchesne *et al.*, 1987; Conn and Tewari, 1991). Phytoalexins were elicited by the pathogen, *B. sorokiniana*, and accumulated in greater amounts in roots of the cultivar Bonanza, which is known to be moderately resistant to the pathogen, than in Samson, which is moderately resistant, and Olli and Galt, which are susceptible. At least two different mechanisms for each cultivar may be involved in disease resistance. However, phytoalexins could not be detected in mycorrhizal barley roots. Therefore, no relationship could be established between phytoalexins elicitation by VA mycorrhizal fungi and reduction in common root rot severity. It is possible that the phytoalexins were indeed elicited but not detected due to low resolution of the technique used. More sensitive detection techniques may be required to detect the phytoalexins in mycorrhizal barley roots.

A third possible mechanism by which VA mycorrhizal fungi could reduce root rot disease is through the regulation of root exudates. Improved phosphorus nutrition with a corresponding reduction in root exudation has previously been reported as a mechanism by which VA mycorrhizal fungi suppress disease (Graham and Menge, 1982). In root exudates of barley cultivars collected over a 14-day period, no significant differences among cultivars in amounts of reducing sugars and amino acids were observed. However, this study was conducted when

phosphorus was supplied in nutrient solution. In addition, no treatments involving inoculation of barley plants with VA mycorrhizal fungi were included. Subsequent studies should also consider growth of these barley cultivars using different phosphorus regimes. The study was a preliminary screening of barley cultivars to determine if differences in total reducing sugars and amino acids were related to resistance to *B. sorokiniana*. Azcon and Ocampo (1981) found that increased levels of total reducing sugars in root exudates were related to increased susceptibility of wheat cultivars to VA mycorrhizal fungi. Future experiments involving the detection of different types of reducing sugars and amino acids may reveal qualitative differences in root exudates among barley cultivars. Schwab *et al.* (1983), however, suggested that differences in specific components of the root exudates are not a reliable indicator of susceptibility to VA mycorrhizal fungi. The suppression of common root rot disease by VA mycorrhizal fungi through changes in root exudation has not been ruled out.

From the results of these studies to investigate the mechanisms by which common root rot disease is reduced by VA mycorrhizal fungi, it is evident that no one mechanism is responsible. Suppression of diseases by mycorrhizal symbionts may be regulated by a number of factors. General resistance of plants to pathogens is usually governed by several mechanisms. An example of this is *Alternaria brassicae* (Berk.) Sacc. in crucifers (Tewari, 1991). Resistance of crucifers to the pathogen involved many factors, including presence of wax on plant surfaces, calcium sequestration, sensitivity of the plant to toxin produced by the pathogen, elicitation of phytoalexins and hypersensitive reaction. The author thus concluded that resistance was multicomponent and layered. The mechanism for suppression of disease by VA mycorrhizal fungi may also be multicomponent and different VA mycorrhizal fungi may reduce disease through different mechanisms. Many studies reporting the interactions between VA mycorrhizal fungi and soil-borne pathogens oversimplify the mechanism involved. The role of phosphorus in this interaction is probably overrated and future work should take a holistic approach.

E. Conclusions

1. Five VA mycorrhizal species were collected from agricultural soils in Alberta. The species were identified as *Glomus mosseae, G. aggregatum, G. pansihalos, G. tenue,* and *Entrophospora infrequens*. This is the first report of *G. pansihalos* from western Canada.

2. Spores of *G. aggregatum* exhibited external proliferation, which is similar to microcyclic sporulation in other fungi.
3. Cell walls of VA mycorrhizal spores are rich in calcium. In addition, one spore wall may have appreciable differences in levels of calcium than another spore wall.
4. No significant differences in susceptibility to indigenous VA mycorrhizal fungi among barley cultivars was observed.
5. Eight barley cultivars inoculated with *Glomus dimorphicum, G. intraradices,* or *G. mosseae* showed differences in susceptibility to the mycorrhizal fungi, depending on the *Glomus* species. Also, differences were observed in the ability of each *Glomus* species to colonize barley roots.
6. Some barley cultivars showed improvement in growth and yield when inoculated with VA mycorrhizal fungi. The host-mycorrhizal fungus genotypes may therefore influence the effectiveness of the symbiosis between the host and VA mycorrhizal species.
7. *Glomus dimorphicum, G. intraradices,* and *G. mosseae* were effective biological control agents of barley common root rot disease, with *G. dimorphicum* being the least effective.
8. Phosphorus played a role in suppression of barley common root rot disease by VA mycorrhizal fungi. However, its role was probably a minor one.
9. Phytoalexins appear to be playing a role in resistance of barley cultivars to common root rot disease, but elicitation of these compounds by VA mycorrhizal fungi was not observed. Due to differences in phytoalexin elicitation among barley cultivars tested, it is believed that different resistance mechanisms to common root rot disease exist.
10. Barley cultivars showed no differences in root exudation of reducing sugars and amino acids. Negative correlations were observed between susceptibility of barley cultivars to *B. sorokiniana* and amount of root exudation. However, no correlations were observed between susceptibility of barley cultivars to VA mycorrhizal fungi and root exudation.

F. Suggestions for future work

1. Systematic study of VA mycorrhizal fungi indigenous to agricultural soils in Alberta, including the collection and pot culturing of species should be undertaken. These fungi thus collected could be utilized by researchers in

Alberta to study the importance of the mycorrhizal symbionts in the agricultural ecosystems.

2. Field studies on practical methods of utilizing indigenous populations of VA mycorrhizal fungi, such as effects of crop rotation on mycorrhizal spore numbers and inoculation trials to re-introduce indigenous species with field crops require attention. Mass production of mycorrhizal fungi for field trials also requires future consideration.
3. Since biological control of soil-borne diseases by VA mycorrhizal fungi is probably mediated by several mechanisms, a holistic approach for studying VA mycorrhizal fungi should be considered. Several mechanisms for suppression of disease need to be examined in individual systems.

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